IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket: LP-1940

Appellants	:	Florian LICHTENBERG et al.	
Serial No.	:	10/501,395	Primary Examiner: Aradhana Sasan
Filed	:	07/15/2004	Group: 1615
Title	:	VIRUCIDAL DISINFECTANT	

APPEAL BRIEF

Mail Stop - Appeal Brief
Patents Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellants have appealed from the final Office Action of January 11, 2008, rejecting all of the pending Claims 1 to 33. Appellants request reversal of the rejections of the pending claims.

The Notice Of Appeal was filed on July 10, 2008.

(1) REAL PARTY IN INTEREST

Lonza AG of Munchensteinerstrasse 38, CH-4002 Basel, Switzerland, is the assignee of the present application, U.S.S.N. 10/501,395. Lonza Group directly and/or indirectly controls Lonza AG.

(2) RELATED APPEALS AND INTERFERENCES

No interferences or appeals are known that are related to the present application.

(3) STATUS OF CLAIMS

This is an appeal from the Final Rejection by the Examiner of Claims 1 to 33, all of the pending claims. No claims have been allowed. Claims 1 to 33 have been rejected.

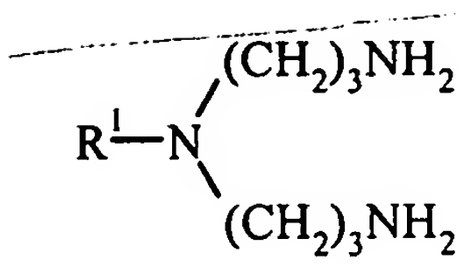
(4) STATUS OF AMENDMENTS

The amendment after final was filed on July 2, 2008, in response to the final Office Action that was mailed on January 11, 2008. The amendment after final rejection (mailed on July 10, 2008) amended Claims 6, 18, 19 and 20. In the Advisory Action mailed on July 28, 2008, the Examiner indicated that, for purposes of appeal, the amendment after final would be entered upon the filing of an appeal. Accordingly, the rejections under 35 USC 112, second paragraph, and 35 USC 101 are now moot. But see page 2 of the Advisory Action that only states that 35 USC 112, second paragraph, is overcome. Appellants' arguments presented herein relate to the rejections under 35 USC 103(a) of Claims 1 to 33 as presented in the final Office Action and the Advisory Action (and to the rejection under 35 USC 101). As amended, Claims 1 to 33 are set out in (8) Claims Appendix.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

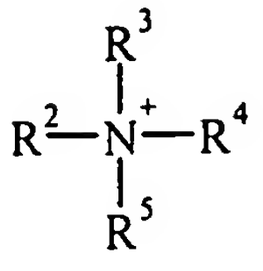
First Grouping: Independent Claim 1 And Dependent Claims 2 To 10 And 12 To 28

The subject matter claimed in the First Grouping [Independent Claim 1 and the listed dependent claims] involves a process of utilizing a disinfectant composition as a virucidal agent. [page 1, line 3 to 5] The virucidal disinfectant composition comprises (a) an amine of formula (Ia) and/or a quaternary ammonium salt of formula (1b):



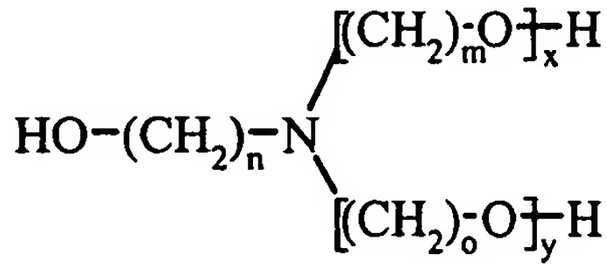
(Ia)

or



(Ib),

and (b) at least one alkanolamine of formula (II):



(II),

[page 1, line 31, to page 2, line 14] In formula (Ia), R^1 is C_{6-18}-alkyl, in formula (Ib), R^2 is benzyl or C_{6-18}-alkyl, R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n = 1-20, R^4 and R^5 independently of one another are C_{1-4}-alkyl, R^6 is hydrogen or unsubstituted or substituted phenyl, and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid, and in formula (II), n and, if present, m and o independently of one another have the value 2 or 3, and x any y independently of one another have the value 0 or 1. [page 1, line 31, to page 2, line 14] Alkanolamine of of formula (II) can be a corresponding salt. [page 2, lines 7 to 14] The mass ratio of compound(s) (I) to compound(s) (II) [i.e., I:II] is 20:1 to 1:20. [page 2, line 14] The virucidal disinfectant composition can also contain water as a sovent, and/or organic solvents, surfactants, complexing agents, fragrances and colorants. [page 3, lines 16 to 23] The virucidal disinfectant composition can be used, for example, for surface

disinfection, instrument disinfection, laundry disinfection, hand disinfection and chemical toilets. [page 3, lines 24 to 32]

SECOND GROUPING: DEPENDENT CLAIMS 11 AND 29 TO 33

The subject matter claimed in the Second Group [dependent Claims 11 and 29 to 33] involves the claimed process of independent Claim 1 of using the virucidal disinfectant composition against polioviruses, picornaviruses and parvovirusus. [page 1, lines 23 to 29]

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The grounds of rejection to be reviewed on appeal are:

(1) Claims 1 to 10 and 12 to 28 have been rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 5,908,854 (McCue et al.).

(2) Claims 11 and 29 to 33 have been rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 5,908,854 (McCue et al.) in view of WO 94/22305 (Bellamy et al.).

(3) Claims 18 and 19 have been rejected under 35 U.S.C. 101 on the ground that such process claims were not process claims because the did not recite any process step. [This ground for rejection may be moot.]

(7) ARGUMENT

Rejection Of Claims I To 10 And 12 To 28 Under 35 U.S.C. 103(a)

Claims 1 to 10 and 12 to 28 have been rejected under 35 U.S.C. 103(a) as being unpatentable over McCue et al. (U.S. Patent No. 5,908,854). Appellants traverse this rejection.

McCue et al. requires the presence of a solvent that is phenoxyalcohols and/or glycol ethers. The Office Action stated however, instant claims have the term "comprising" which allows for the presence of these solvents. The burden of proof is upon the Examiner to show that the addition of such chemicals would not destroy appellants' claimed process.

The Examiner has cited Bellamy et al. in another obviousness rejection in this case. All of the teachings in Bellamy et al. also have to be considered under Section 103(a). McCue et al. is not combinable in the search for appellants' claimed invention and, without the required solvents of McCue et al., the McCue et al. system/invention is ineffective and destroyed. McCue et al. cannot be used by itself because Bellamy et al. is present and its negative effect thereon cannot be ignored under Section 103(a).

The Office Action stated that this is not found persuasive because McCue et al. clearly states that water may be included to dilute the compositions (McCue et al., col. 4, lines 43 to 45), and thus, one does not destroy the efficacy of the composition. The Examiner's statement is not factual because different chemical composition are involved. Speculation does not suffice under Section 103(a).

Appellants claim a process of using specifically-defined disinfectant compositions as a virucidal agent and the Examiner has incorrectly attempted to generify appellants' specific process.

The statement in the Office Action that "the disinfectant composition comprises (a) an amine (Ia) or a quaternary ammonium compound (Ib) and (b) at least one alkanolamine (11), in the mass ratio 1:11 of 20:1 to 1:20" is an incorrect attempt to

generify appellants' specific ingredients/chemicals. The specifically defined compounds are required by appellants' claims, not the generic approach taken by this rejection.

The Office Action stated that, however, the substituents in appellants' claims (e.g. R^1-R^5 can be alkyl) lead to a generic quaternary ammonium compound and an alkanolamine.

The Office Action stated that McCue et al. teaches that many quaternary ammonium compositions "exhibit broad spectrum bactericidal, fungicidal and virucidal activity..." (Col. 1, lines 11 to 16). This statement does not establish that McCue et al.s composition has any virucidal activity. (Other statements of the Examiner confirm this point.) McCue et al. only teaches that its composition is mycobactericidal.

The Office Action stated that the property of virucidal activity is implicit to the composition comprising a quaternary ammonium compound even though the reference fails to use the composition as a virucide. Appellants traverses this statement as being factually incorrect and mere spectulation.

The Office Action stated that appellants have failed to show that the composition of McCue et al. is not virucidal. The appellants do not have the burden of proving such negative. However, the Examiner has failed to show that one ordinarily skilled in the art would believe that McCue et al. even implies that its composition is virucidal.

The Office Action stated that appellants argue that no prior art teaching any virucidal activity of quaternary disinfectant compositions is specifically cited in McCue et al. and the expression "quaternary disinfectant composition" merely means a composition containing a quaternary ammonium compound. Appellants' assertion is correct.

The Office Action stated that appellants argue that nowhere in McCue et al. is there any teaching that the alleged virucidal activity of those unspecified compositions is caused by the quaternary ammonium compound and not by another ingredient, e.g., by a virucidal agent known in the art. Appellants' assertion is correct.

The Office Action stated that appellants argue that McCue et al. is completely silent on the virucidal activity; that McCue et al. direct one ordinarily skilled in the art away from appellants' claimed invention, and that McCue et al. is not a relevant reference in the quest for appellants' claimed process. Appellants' assertion is correct.

The Office Action stated that appellants also argue that there is no suggestion or teaching to use the McCue et al. compositions against viruses or that they would be virucidal.

The Office Action stated that although McCue et al. does not expressly teach the use of the composition against viruses, this does not mean that the composition of McCue et al. is not effective against viruses. Appellants' assertion is correct.

The Office Action stated that, conversely, it implies that the virucidal effect is easier than the mycobactericidal effect. Appellants traverse this statement as being factually incorrect and mere speculation. The Examiner has not supplied any facts supporting his spectulation.

The Office Action stated that a person having ordinary skill in the art at the time the invention was made would have arrived at the conclusion that using the McCue et al. composition would have a virucidal effect and also have a broad spectrum bactericidal and fungicidal effect. Appellants traverse this statement. The Examiner does not know what one ordinarily skilled in the art would have concluded because the

Examiner has not factually determined in the record what is the level of ordinary skill in the art and so does not know anything about one ordinarily skilled in the art.

The Office Action stated that appellants argue that the McCue et al. teaching of an alkanolamine compound, such as "mono-, di- or tri-ethanolamine" is of no meaning since McCue et al. directs away from virucidal compositions. Appellants' assertion is correct.

The Office Action stated that therefore, the argument that McCue et al.'s teaching of an alkanolamine is "of no meaning" is unclear. McCue et al. does not teach or imply that its composition is virucidal, hence the basis for "of no meaning" under Section 103(a) as regards appellants' claims.

The Office Action stated that Section 2144.05 of MPEP states: "Generally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical. '[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation.' In re Aller, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)". This quotation is meaningless because it is not based upon the controlling, mandatory basic factual inquiries and determinations first being made as a framework.

Section 103(a) deals with one ordinarily skilled in the art, not one skilled in the art. The Office Action stated that the Examiner recognizes that Section 103(a) deals with a person having ordinary skill in the art. Then the Examiner know that the obviousness rejections fail.

The Office Action stated that appellants argue that this rejection is faulty on its face because the Examiner has not factually resolved, as is mandatory in the record, the level of ordinary skill in the art. Appellants' assertion is correct.

The Office Action stated that, however, the Examiner considered:

(1) The scope and content of the prior art (McCue et al. teaches that many quaternary ammonium compositions "exhibit broad spectrum bactericidal, fungicidal and virudical activity..." (Col. 1, lines 11 to16),

(2) The differences between the claimed invention and the prior art (McCue et al. does not expressly teach that the process of utilizing the composition against parvoviruses, picornaviruses and polioviruses),

(3) The level of ordinary skill in the pertinent art (a person having ordinary skill in the art would know how to titrate the composition in order to maintain the disinfecting efficacy), and

(4) Objective evidence relevant to the issue of obviousness (McCue et al. teaches that the compositions may be applied to a surface which is need of disinfectant (Col. 5, lines 54 to 56).

These are not the factual inquiries and determinations required by the Graham and KSR decisions and Patent Office policy. Furthermore, the fact considered, the determination reasons, etc., are missing from the record – the Patent Office requires that the record be complete and written. With the elements and facts missing, appellants have been deprived of due process in that they have been denied the record upon to ascertain if they agree or disagree with the Examiner, and to oppose the Examiner's determinations if such be the case.

The Office Action stated that appellants argue that the McCue et al. teachings of didecyl dimethyl ammonium chloride as being obvious to one skilled in the art as a quaternary ammonium salt is meaningless – wrong standard. The Office Action stated that appellants do not explain why this teaching of a quaternary ammonium salt is meaningless and wrong. The wrong standard is "one skilled in the art".

The Office Action states that appellants argue that the statement of the McCue et al. teaching of an ethanolamine such as mono-, di- or tri-ethanolamine as being obvious to one skilled in the art as an alkanolamine compound is in error and the Examiner has not factually resolved the ordinary level of skill in the art, hence he knows nothing about one ordinarily skilled in the art or what would be obvious to such to such a person. The Examiner has not made a factual inquiry and factually determined in the record the level of ordinary skill in the art. If the Examiner thinks that he has done such, please point where for the Board and appellants.

The Office Action stated that however, as shown above, the Examiner determined the ordinary level of skill in the art in and since McCue et al. teaches an alkanolamine, the rejection will be maintained. This statement is incorrect. The Examiner has not made the factual inquiry and determination of the level of ordinary skill in the art, and has not placed the supporting facts, analysis, determination, etc., in the record.

The Office Action stated that appellants argue that dependent claims are unobvious since the independent claim is unobvious. Appellants are correct. The Office Action stated that, however, the demonstration of the consideration of the

Graham factual inquiry is stated above. The Examiner did not consider the Graham factors above.

The Office Action stated that since McCue et al. teaches the use of the composition to disinfect instruments, apparatuses and in a wide variety of environments which may benefit from a disinfecting effect (Col. 4, lines 51 to 53), a person having ordinary skill in the art at the time the invention was made would a have found it obvious to use the composition for laundry disinfection, hand disinfection, and in chemical toilets (which are all environments that may benefit from a disinfecting effect). The Examiner has no basis for saying what would be obvious to one ordinarily skilled in the art. This obviousness rejection has been shown to not be in accord with Patent Office policy by failing to factually determine in the record the level of ordinary skill in the art and set out in the record the facts considered, analysis, etc., in the subject mandatory factual inquiry and determination. This obviousness rejection has also not complied with the requirements of the Supreme Court's Graham and KSR decisions. Appellants have, accordingly, been denied the right to consider the factual and other bases for this obviousness rejection and to oppose this rejection based on such facts, analysis, etc., and errors concerning such facts, analysis, etc., if such be the case.

The Office Action stated that the invention as a whole was prima facie obvious to one of ordinary skill in the art at the time the invention was made, as evidenced by the references, especially in the absence of evidence to the contrary. Appellants traverse this statement. The Examiner has not established any case of prima facie obviousness of appellants' claims to one ordinarily skilled in the art because the Examiner has not

factually, in the record, determined the level of ordinary skill in the art (so he does not know what would be obvious to one ordinarily skilled in the art).

The Office Action stated that McCue et al. teaches that many quaternary ammonium compositions "exhibit broad spectrum bactericidal, fungicidal and virucidal activity.:." (Col. 1, lines 11 to 16). The Office Action stated that, therefore, a person having ordinary skill in the art would find it obvious that the instant invention would be effective as a virucidal agent. This statement is also in error as the Examiner does not known what would be obvious to one of ordinary skill in the art.

The Examiner has failed to factually determine, in the record, the mandatory factual inquiry of the level of ordinary skill in the art, hence the Examiner has not defined one ordinarily skilled in the art. This missing determination means that the substantive requirements of the Graham and KSR decisions and Patent Office policy have not been met and that the Section 103(a) rejection are defective on their face.

Rejection Of Claims 11 and 29 To 33

Claims 11 and 29 to 33 have been rejected under 35 U.S.C. 103(a) as being unpatentable over McCue et al. (U.S. Patent No. 5,908,854), in view of Bellamy et al. (WO 94/22305). Appellants traverse this rejection.

Appellants' disinfectant process is particularly and unexpectedly effective parvoviruses, picornaviruses and polioviruses. This shown by appellants' data in appellants' published U.S. Patent Application Publication No. 20050089496 A1 and WO 03/059062 A1.

McCue et al. does not teach the use of its composition against any viruses.

The prior Office Action stated that, although McCue et al. does not expressly teach the use of the composition against viruses, conversely, it implies that the virucidal effect is easier than the mycobactericidal effect. This statement is mere speculation. Section 103(a) requires facts, not speculation. As the Examiner has admitted, McCue et al. does not specifically teach the use of its composition against paraoviruses or picornaviruses or polioviruses – he admits more broadly that McCue et al does not expressly teach the use of its composition against viruses.

The Office Action incorrectly stated that a person having ordinary skill in the art at the time the invention was made would have arrived at the conclusion that using the McCue et al. composition would have a virucidal effect and also have a broad spectrum bactericidal and fungicidal effect. The Examiner has not factually determined (as required by the Supreme Court and Patent Office policy) in the record the level of ordinary skill in the art so the Examiner does not know anything about one ordinarily skilled in the art or what such a person would due, conclude, etc. The Examiner did not make the substantive legal requirement of make such a factual inquiry and factual determination. This obviousness rejection is invalid.

The prior Office Action stated that appellants argue that Bellamy et al. does not cure the defects of McCue et al.

The prior Office Action stated that Bellamy et al. expressly teaches a virucidal composition suitable as a hospital disinfectant, comprising alkaline material and an alktl quaternary nitrogen salt, effective for killing polioviruses and disinfecting heat sensitive medical instruments (Abstract). This statement is factually incorrect. Bellamy et al. is specifically restricted to "alkaline material selected from alkali metal carbonates and

alkali metal hydroxides" – see the definition of the Bellamy et al. invention on page 2 of Bellamy et al. Since all of the teachings of a reference must be considered under Section 103(a), the combination of Bellamy et al. and McCue et al. results in many different processes. The Examiner has no basis of record to use the alkanolamine of McCue et al. instead of the alkali metal carbonate or hydroxide of Bellamy et al. The invention of Bellamy et al. is destroyed by trying to insert a different chemical in place of a major chemical in the Bellamy et al. composition. Therefore, the combination of McCue et al. and Bellamy et al. does not result in appellants' claimed process.

The prior Office Action stated that therefore, to a person having ordinary skill in the art, Bellamy et al. cures the deficiency of McCue et al. The Examiner cannot any statement regarding one ordinarily skilled in the art because he has not factually determined in the record the level of ordinary skill in the art.

The prior Office Action stated: that appellants argue that the compositions of the instant application do not contain ethoxylated alkyphenols; and that, however, instant claims have the term "comprising" which allows for the presence of these solvents. Appellants disagree because the Examiner has not factually shown in the record that inclusion of such chemicals in appellants' process would not destroy it or have an adverse effect thereon. The Examiner has the burden of proof.

Appellants advance that a person of ordinary skill in the art will recognize that the last part of the Bellamy et al. statement at col. 4, lines 38 to 40, is wrong scientifically and factually since viruses do not have a cell wall. The Office Action stated that the Examiner recognizes the error in the McCue et al. reference regarding the fact that viruses do not have cell walls – the Examiner refers to the wrong reference.

Independent Claim 1 is unobvious so these dependent claims are also unobvious and that one ordinarily skilled in the art would not consider Bellamy et al. to be a relevant reference. One ordinarily skilled in the art has no basis for paying no attention to core assertions of the later McCue et al. You cannot destroy one reference to save another.

The Office Action stated that, however, although McCue et al. does not expressly teach the use of the composition against viruses, this does not mean that the composition of McCue et al. is not effective against viruses. Appellants that Section 103(a) requires facts, not mere speculation.

The Office Action stated that a person having ordinary skill in the art at the time the invention was made would have arrived at the conclusion that using the McCue et al. composition would have a virucidal effect and also have a broad spectrum bactericidal and fungicidal effect. The Examiner does not know what conclusion one ordinarily would have arrived at.

The Office Action stated that appellants argue that regarding the combination of the use of the disinfectant composition taught by McCue et al. with the virucidal (particulary against poliovirus) Bellamy et al. composition, the Examiner cannot make any statement re one ordinarily skilled in the art. Appellants are correct because the Examiner has not factually determined (as is mandatory) the level of ordinary skill in the art.

The Office Action stated that appellants argue that both reference are not relevant and McCue et al. directs away from appellants' claimed invention. Appellants' assertion is correct. The Office Action incorrectly stated that' however, since the

question of obviousness was resolved on the basis of the Graham factual inquiries, the Examiner can make statements about a person having ordinary skill in the art (see discussion above). The Examiner has not factually determined in the record the level of ordinary skill in the art. The Patent Office rules require that such a factual inquiry and determination have to be in the record, but they are not in the record.

The Office Action stated: that, regarding the combination of references, all the claimed elements are found in McCue et al. and Bellamy et al. and one skilled in the art could have combined the elements and the combinations would have yielded predictable results; and see KSR International Co. v. Teleflex Inc., 550 U.S.-, 82 USPQ2d 1385 (2007). The Examiner has conclusively shown why this obviousness rejection is invalid and fatally defective. "...one skilled in the art has nothing to do with Section 103(a).

The Office Action stated that McCue et al. does not specifically teach the use of the composition against parvoviruses, picornaviruses, or polioviruses.

The Office Action stated that Bellamy et al. teach an aqueous virucidal composition suitable as hospital disinfectant, comprising alkaline material and an alkyl quaternary nitrogen salt, effective for killing polioviruses and disinfecting heat sensitive medical instruments (Abstract).

The Office Action stated that Bellamy et al. uses dialkyl dimethyl quaternary ammonium chloride as an example (Page 5, lines 15 to 17).

The Office Action stated that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the composition comprising a germicidal quaternary ammonium compound, and an alkanolamine as a disinfectant, as

suggested by McCue et al., combine it with the virucidal composition, as suggested by Bellamy et al., and produce the instant invention.

The Office Action stated that one of ordinary skill in the art would have been motivated to do this because Bellamy et al. demonstrates the effectiveness of the anti-viral disinfectant composition, particularly against poliovirus (page 8, lines 1 to 7). The Examiner has no basis of record for making any statement regarding one ordinarily skilled in the art.

The Office Action stated that, regarding instant Claims 11 and 29 to 33, the limitation of the virucidal agent utilized against paroviruses, picornaviruses or polioviruses would have been obvious to one of ordinary skill in the art over the effectiveness of the anti-viral disinfectant composition, particularly against poliovirus as taught by Bellamy et al. (page 8, lines 1 to 7). Appellants traverse the statement – on several bases including the one that the Examiner does not know anything about one ordinarily skilled in the art because the Examiner has not made the mandatory factual inquiry and determination in the record of the level of ordinary skill in the art.

Appellants also point out that the Examiner did not determine the mandatory substantive factual inquiries in the Graham and KSR decisions and required by Patent Office policy.

Rejection Of Claims 18 And 19 Under 35 U.S.C. 101

The Section 101 rejection of Claims 18 and 19 should have been withdrawn because each of such dependent claims are dependent ultimately upon independent process Claim 1.

Appellants request reversal of the final rejections of the claims and allowance of the claims.

(8) Claims Appendix

Claims Appendix (8), that contains a copy of the claims on appeal, is attached hereto.

(9) Evidence Appendix

Evidence Appendix (9), that contains a copy of evidence, is attached hereto.

(10) Related Proceedings Appendix

Related Proceedings Appendix (10) is attached hereto.

(11) Miscellaneous Matters

There is nothing included in Related Proceeding Appendix (10) of this Appeal Brief.

A check in the amount of $ 540.00 for this Appeal Brief fee is enclosed herewith.

The Commissioner for Patents is hereby authorized to charge any additional fees, or credit any overpayments, to Deposit Account No. 06-1110.

This Appeal Brief is being submitted in executed triplicate.

Appellants request reversal of the final rejections of and allowance of Claims 1 to 33.

Respectfully submitted,

December 9, 2008
Date

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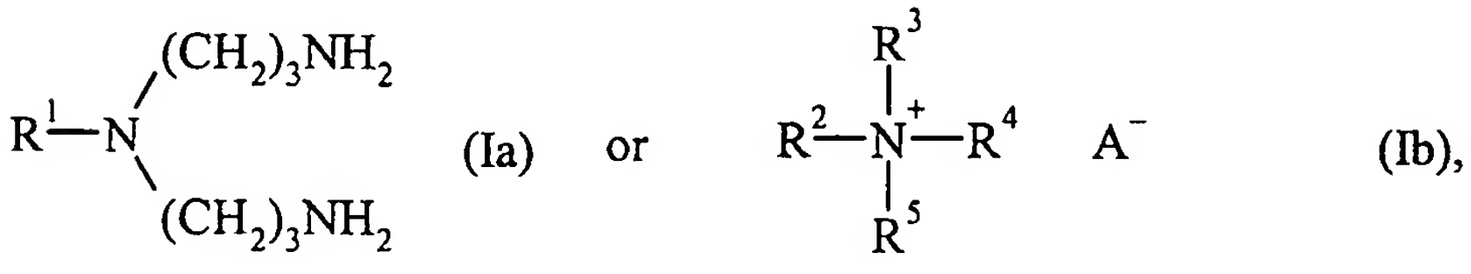
(8) CLAIMS APPENDIX

1. A process of utilizing a disinfectant composition comprising:

a) an amine and/or quaternary ammonium salt of the general formula:



(Ia) or (Ib),

where R^1 is C_{6-18}-alkyl,

R^2 is benzyl or C_{6-18}-alkyl,

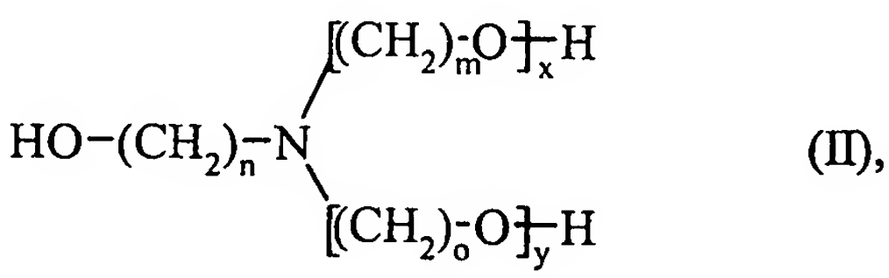
R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n = 1-20,

R^4 and R^5 independently of one another are C_{1-4}-alkyl,

R^6 is hydrogen or unsubstituted or substituted phenyl,

and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

b) at least one alkanolamine of the general formula:



(II),

where n and, if present, m and o independently of one another have the value 2 or 3,

and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio I:II of 20:1 to 1:20, as a virucidal agent.

2. The process according to Claim 1, wherein the amine or quaternary ammonium salt is selected from the group consisting of N,N-bis-(3-aminopropyl)dodecylamine, N,N-bis(3-aminopropyl)octylamine, didecyldimethylammonium salts, dioctyldimethylammonium salts, octyldecyldimethyl-ammonium salts, cocoalkyldimethylbenzylammonium salts and benzyldimethyloxoethylammonium salts and mixtures of these compounds.

3. The process according to Claim 1, wherein the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.

4. The process according to Claim 1, wherein the mass ratio I:II is between 1:5 and 5:1.

5. The process according to Claim 1, wherein the disinfectant composition comprises water as solvent.

6. The process according to Claim 1, wherein the disinfectant composition additionally comprises one or more auxiliaries selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

7. A process according to Claim 1, wherein the virucidal agent of Claim 1 is utilized for surface disinfection and instrument disinfection.

8. A process according to Claim 1, wherein the virucidal agent of Claim 1 is utilized for laundry disinfection.

9. A process according to Claim 1, wherein the virucidal agent of Claim 1 is utilized for hand disinfection.

10. A process according to Claim 1, wherein the virucidal agent of Claim 1 is utilized for chemical toilets.

11. A process wherein the virucidal agent of Claim 1 is utilized against parvoviruses, picornaviruses or polioviruses.

12. The process according to Claim 2, wherein the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.

13. The process according to Claim 2, wherein the mass ratio I:II is between 1:5 and 5:1.

14. The process according to Claim 3, wherein the mass ratio I:II is between 1:5 and 5:1.

15. The process according to Claim 12, wherein the mass ratio I:II is between 1:5 and 5:1.

16. The process according to Claim 2, wherein the disinfectant composition comprises water as solvent.

17. The process according to Claim 12, wherein the disinfectant composition comprises water as solvent.

18. The process according to Claim 15, wherein the disinfectant composition comprises water as solvent.

19. The process according to Claim 2, wherein the disinfectant composition additionally comprises one or more auxiliaries selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

20. The process according to Claim 18, wherein the disinfectant composition additionally comprises one or more auxiliaries selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

21. A process wherein the virucidal agent according to Claim 2 is utilized for surface disinfection and instrument disinfection.

22. A process wherein the virucidal agent according to Claim 20 is utilized for surface disinfection and instrument disinfection.

23. A process wherein the virucidal agent according to Claim 2 is utilized for laundry disinfection.

24. A process wherein the virucidal agent according to Claim 20 is utilized for laundry disinfection.

25. A process wherein the virucidal agent according to Claim 2 is utilized for hand disinfection.

26. A process wherein the virucidal agent according to Claim 20 is utilized for hand disinfection.

27. A process wherein the virucidal agent according to Claim 2 is utilized for chemical toilets.

28. A process wherein the virucidal agent according to Claim 20 is utilized for chemical toilets.

29. A process wherein the virucidal agent according to Claim 2 is utilized against parvoviruses, picornaviruses or polioviruses.

30. A process wherein the virucidal agent according to Claim 22 is utilized against parvoviruses, or picornaviruses or polioviruses.

31. A process wherein the virucidal agent according to Claim 24 is utilized against parvoviruses, or picornaviruses or polioviruses.

32. A process wherein the virucidal agent according to Claim 26 is utilized against parvoviruses, or picornaviruses or polioviruses.

33. A process wherein the virucidal agent according to Claim 28 is utilized against parvoviruses, or picornaviruses or polioviruses.

(9) EVIDENCE APPENDIX

A copy of WO 03/059062 A1 is enclosed, which is a published copy of appellants' International Application No. PCT/EP03/00378. It is a published reference. This document was filed with the U.S. application on filing date of July 15, 2004. The enclosed copy of the Transmittal Letter and the date stamped postcard show that such reference was received and is part of the record (i.e., entered).

Also enclosed is a copy of appellants' U.S. Patent Application Publication 2005/0089496 A1, which is a published reference. Enclosed is a copy of the Notice Of Publication Of Application.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

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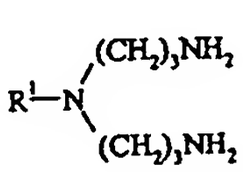
Erklärungen gemäß Regel 4.17:

— *hinsichtlich der Berechtigung des Anmelders, ein Patent zu beantragen und zu erhalten (Regel 4.17 Ziffer ii) für die folgenden Bestimmungsstaaten AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,*

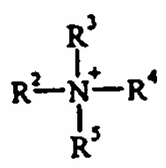
[Fortsetzung auf der nächsten Seite]

(54) Title: VIRUCIDAL DISINFECTANT

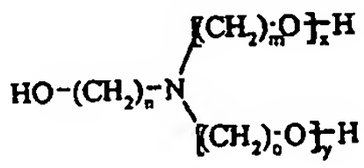
(54) Bezeichnung: VIRUZIDE DESINFEKTIONSMITTEL



(Ia)



(Ib)



(II)

(57) Abstract: Disclosed is the use of disinfectant compositions as a virucide, particularly against polio viruses, containing a) at least one amine and/or quaternary ammonium salt of general formula (Ia) or (Ib), in which R^1 represents C_{6-18} alkyl, R^2 represents benzyl or C_{6-18} alkyl, R^3 represents C_{1-18} alkyl or $-[(CH_2)_2-O]_nR^6$, with n = 1-20, R^4 and R^5 independently represent C_{1-4} alkyl, R^6 represents hydrogen or optionally substituted phenyl, and A^- represents a monovalent anion or an equivalent of a polyvalent anion of an inorganic or organic acid; and b) at least one alkanolamine of general formula (II), in which n and optionally m and o independently have the value 2 or 3 and x and y independently have the value 0 or 1, or a corresponding salt; at a mass ratio I:II of 20:1 to 1:20. Said compositions also have good bactericidal and fungicidal qualities, even when used at low concentrations.

(57) Zusammenfassung: Beschrieben wird die Verwendung von Desinfektionsmittelzusammensetzungen, die a) wenigstens ein Amin und/oder quartäres Ammoniumsalz der allgemeinen Formel (Ia) oder (Ib), worin R^1 C_{6-18}-Alkyl, R^2 Benzyl oder C_{6-18}-Alkyl, R^3 C_{1-18}-Alkyl oder $-[(CH_2)_2-O]_nR^6$ mit n = 1-20, R^4 und R^5 unabhängig voneinander C_{1-4}-Alkyl, R^6 Wasserstoff oder gegebenenfalls substituiertes Phenyl und A^- ein einwertiges Anion oder ein Äquivalent eines mehrwertigen Anions einer anorganischen oder organischen Säure bedeutet; und b) wenigstens ein Alkanolamin der allgemeinen Formel (II), worin n und, soweit vorhanden, m und o unabhängig voneinander den Wert 2 oder 3 und x und y unabhängig voneinander den Wert 0 oder 1 haben, oder ein entsprechendes Salz; im Massenverhältnis I:II von 20:1 bis 1:20 enthalten, als Viruzid, insbesondere gegen Polioviren. Die Zusammensetzungen besitzen ausserdem gute bakterizide und fungizide Wirksamkeit auch bei geringen Anwendungskonzentrationen.

WO 03/059062 A1

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO-Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

— *Erfindererklärung (Regel 4.17 Ziffer iv) nur für US*

Veröffentlicht:

— *mit internationalem Recherchenbericht*

— *vor Ablauf der für Änderungen der Ansprüche geltenden Frist; Veröffentlichung wird wiederholt, falls Änderungen eintreffen*

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

Viruzide Desinfektionsmittel

Die Erfindung betrifft die Verwendung von synergistischen Desinfektionsmittelzusammensetzungen auf Basis von Aminen und/oder quartären Ammoniumsalzen als viruzide Mittel, insbesondere gegen Polioviren.

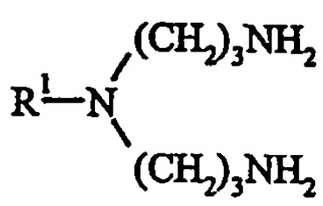
Es sind zahlreiche Desinfektions- und Konservierungsmittelzusammensetzungen auf Basis von Aminen und/oder quartären Ammoniumsalzen bekannt. Diese weisen jedoch im allgemeinen, insbesondere bei höheren Verdünnungen, eine unbefriedigende Wirksamkeit gegen Pilze wie z. B. *Aspergillus niger* und Viren (insbesondere gegen hochresistente Viren wie z. B. Polioviren) auf.

Aufgabe der vorliegenden Erfindung war daher die Bereitstellung von Desinfektionsmittelzusammensetzungen auf Basis von Aminen und/oder quartären Ammoniumsalzen, welche auch bei hoher Verdünnung eine gute Wirksamkeit gegen Pilze und insbesondere gegen Viren aufweisen.

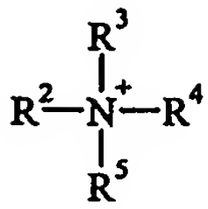
Erfindungsgemäss wird diese Aufgabe durch die Verwendung nach Patentanspruch 1 gelöst.

Die ältere Anmeldung PCT/EP 01/10754 (publiziert als WO 02/23990 A1) beschreibt Desinfektionsmittelzusammensetzungen auf Basis von Aminen und/oder quartären Ammoniumsalzen und Alkanolaminen und deren fungizide Eigenschaften.
Es wurde nun überraschend gefunden, dass solche Desinfektionsmittelzusammensetzungen auch ausgeprägte viruzide Eigenschaften und insbesondere auch eine gute Wirksamkeit gegen hochresistente Viren wie Polioviren aufweisen. Ebenfalls wirksam sind sie gegen andere Picornaviren wie beispielsweise ECHO-Viren oder entsprechende tierpathogene Viren wie ECBO-Viren sowie gegen Parvoviren wie beispielsweise Canine Parvovirus.

Die Zusammensetzungen umfassen Amine und/oder quartäre Ammoniumsalze der allgemeinen Formel



(Ia) oder



(Ib),

worin R^1 C_{6-18}-Alkyl,

R^2 Benzyl oder C_{6-18}-Alkyl

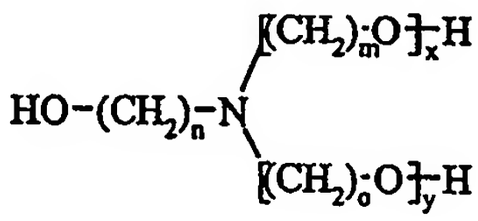
R^3 C_{1-18}-Alkyl oder $-[(CH_2)_2-O]_nR^6$ mit n = 1–20

R^4 und R^5 unabhängig voneinander C_{1-4}-Alkyl

R^6 Wasserstoff oder gegebenenfalls substituiertes Phenyl

und A^- ein einwertiges Anion oder ein Äquivalent eines mehrwertigen Anions einer anorganischen oder organischen Säure bedeutet;

und wenigstens ein Alkanolamin der allgemeinen Formel



(II),

worin n und, soweit vorhanden, m und o unabhängig voneinander den Wert 2 oder 3 und x und y unabhängig voneinander den Wert 0 oder 1 haben, oder einem entsprechenden Salz; im Massenverhältnis I:II von 20:1 bis 1:20.

Unter Alkyl sind hier und im folgenden jeweils lineare oder verzweigte Alkylgruppen der angegebenen Kohlenstoffzahlen zu verstehen, vorzugsweise jedoch lineare Alkylgruppen und besonders bevorzugt solche mit gerader Zahl von Kohlenstoffatomen. Insbesondere sind hierunter auch die von natürlichen Rohstoffen abgeleiteten Homologengemische wie beispielsweise „Kokosalkyl“ zu verstehen.

Unter substituiertem Phenyl sind insbesondere mit einer oder mehreren C_{1-8}-Alkylgruppen und/oder Chloratomen substituierte Phenylgruppen zu verstehen.

Als Anion A^- eignen sich grundsätzlich alle anorganischen oder organischen Anionen, insbesondere Halogenid wie beispielsweise Chlorid oder Bromid oder Anionen niedriger

Carbonsäuren wie beispielsweise Acetat, Propionat oder Lactat.

Das Amin bzw. quartäre Ammoniumsalz (Ia/Ib) ist vorzugsweise *N*,*N*-Bis(3-aminopropyl)-dodecylamin, *N*,*N*-Bis(3-aminopropyl)octylamin, ein Didecyl-dimethylammoniumsalz, Dioctyl-dimethylammoniumsalz, Octyl-decyl-dimethylammoniumsalz, Dikokosalkyl-dimethylammoniumsalz, Kokosalkyl-dimethyl-poly(oxyethyl)ammoniumsalz, Dikokosalkyl-methyl-poly(oxyethyl)ammoniumsalz, Decyl-dimethyl-poly(oxyethyl)ammoniumsalz, Didecyl-methyl-poly(oxyethyl)ammoniumsalz, Octyl-dimethyl-poly(oxyethyl)ammoniumsalz, Dioctyl-methyl-poly(oxyethyl)ammoniumsalz, Kokosalkyl-dimethyl-benzylammoniumsalz, Benzyl-dodecyl-dimethylammoniumsalz oder Benzyl-dimethyl-poly(oxyethyl)ammoniumsalz oder ein Gemisch von zweien oder mehreren dieser Verbindungen. Besonders gute Ergebnisse wurden mit Didecyl-dimethylammoniumsalzen erzielt.

Als Alkanolamine (II) eignen sich grundsätzliche alle Ethanol- und Propanolamine, insbesondere Monoethanolamin, Diethanolamin, Triethanolamin und 3-Amino-1-propanol. Es liegt selbstverständlich auch im Rahmen der Erfindung, Gemische der genannten Verbindungen einzusetzen. Besonders gute Ergebnisse wurden mit den Verbindungen mit primärer Aminogruppe erhalten, nämlich mit Monoethanolamin und 3-Amino-1-propanol.

Das Massenverhältnis von Amin (Ia) bzw. quartärem Ammoniumsalz (Ib) zu Alkanolamin (II) liegt vorzugsweise im Bereich von 1:5 bis 5:1.

Die erfindungsgemäss verwendeten Desinfektionsmittelzusammensetzungen enthalten vorzugsweise Wasser als Lösungsmittel, gegebenenfalls in Kombination mit einem organischen Lösungsmittel.

Vorzugsweise enthalten die erfindungsgemäss verwendeten Desinfektionsmittel-zusammensetzungen noch einen oder mehrere Hilfsstoffe aus der Gruppe bestehend aus organischen Lösungsmitteln, Tensiden, Komplexbildnern, Duftstoffen und Farbstoffen.

Eine bevorzugte Verwendung der Desinfektionsmittelzusammensetzungen ist die Flächen- und Instrumentendesinfektion.

Weitere bevorzugte Verwendungsgebiete sind die Wäschedesinfektion und die Händedesinfektion.

Eine weitere bevorzugte Verwendung der Desinfektionsmittelzusammensetzungen ist der Einsatz in chemischen Toiletten wie beispielsweise an Bord von Flugzeugen und Fahrzeugen.

Die folgenden Beispiele verdeutlichen die Ausführung der Erfindung, ohne dass darin eine Beschränkung auf die beschriebenen Ausführungsformen zu sehen ist. Alle Mengenangaben sind, soweit nicht anders angegeben, in Massen-%. Als Testkeim wurde jeweils *Aspergillus niger* ATCC 16404 eingesetzt. Die Wirksamkeit wurde, soweit nichts anderes angegeben ist, nach dem in CEN 1275 spezifizierten Verfahren bestimmt.

Beispiel 1

Es wurde eine Desinfektionsreinigerformulierung (Konzentrat) hergestellt aus:

5,0% Didecyldimethylammoniumchlorid (50%ige Lösung)
2,0% *N,N*-Bis(3-aminopropyl)dodecylamin
5,0% Monoethanolamin
5,0% Genapol® T250 (Talgfettalkoholpolyglycolether, 25 mol Ethylenoxid)
0,5% Natriummetasilicat
0,5% Natriumcarbonat
2,0% Methylglycindiessigsäure-Trinatriumsalz (Trilon® M; 40%ige Lösung)
Wasser ad 100%

Die Wirksamkeit wurde mit einer Verdünnung (1 Teil Konzentrat, 99 Teile Wasser) bei 20 °C und 15 min Kontaktzeit bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war 4,1.

Vergleichsbeispiel 1

Es wurde wie in Beispiel 1 verfahren, jedoch mit dem Unterschied, dass das Monoethanolamin durch die gleiche Menge Wasser ersetzt wurde. Unter den gleichen Testbedingungen war die Formulierung praktisch unwirksam.

Beispiel 2

Es wurde eine Desinfektionsmittelformulierung (Konzentrat) hergestellt aus:

4,9% *N,N*-Bis(3-aminopropyl)dodecylamin
4,0% Monoethanolamin
2,0% Genapol® T250 (Talgfettalkoholpolyglycolether, 25 mol Ethylenoxid)
5,0% Hostapur® SAS 30 (C_{13-17} sekundäre *n*-Alkansulfonsäure, Natriumsalz)
2,0% Ethylendiamintetraessigsäure-Tetranatriumsalz (40%ige Lösung)
0,7% Ethylendiamintetraessigsäure
Wasser ad 100%

Die Wirksamkeit wurde mit einer Verdünnung (1 Teil Konzentrat, 199 Teile Wasser) bei 20 °C und 15 min Kontaktzeit bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war 4,3.

Beispiel 3

Es wurde eine Desinfektionsmittelformulierung (Konzentrat) hergestellt aus:

4,2% *N,N*-Bis(3-aminopropyl)dodecylamin
2,0% Didecyl-methyl-poly(oxyethyl)ammoniumpropionat (BARDAP 26)
4,0% Monoethanolamin
2,0% Genapol® T250 (Talgfettalkoholpolyglycolether, 25 mol Ethylenoxid)
5,0% Hostapur® SAS 30 (C_{13-17} sekundäre *n*-Alkansulfonsäure, Natriumsalz)

2,0% Ethylendiamintetraessigsäure-Tetranatriumsalz (40%ige Lösung)

0,7% Ethylendiamintetraessigsäure

4,0% Butyldiglycol

Wasser ad 100%

Die Wirksamkeit wurde mit einer Verdünnung (1 Teil Konzentrat, 199 Teile Wasser) bei 20 °C und 15 min Kontaktzeit bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war >4,4.

Zusätzlich wurde die Wirksamkeit noch nach dem in CEN 1650 spezifizierten Verfahren bei einer Kontaktzeit von 15 min, einer Konzentration von 1,0%, einer Wasserhärte von 30 °fH und einer organischen Belastung von 0,3% Albumin bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war >4,4.

Beispiele 4–19

Es wurden wässrige Lösungen aus 0,5% Alkanolamin (II) und 0,25% Amin bzw. quartäres Ammoniumsalz (Ia/Ib) hergestellt und nach dem in CEN 1275 spezifizierten Verfahren gestestet. Die Ergebnisse sind in der folgenden Tabelle 1 zusammengefasst.

Tabelle 1

Beispiel Nr.	Amin/Ammoniumsalz	Alkanolamin	log_{10} Keimreduktion
4	Dimethyl-dioctyl-ammoniumchlorid	Monoethanolamin	4,3
5	dto.	Diethanolamin	4,0
6	dto.	Triethanolamin	3,6
7	dto.	3-Amino-1-propanol	4,2
8	Didecyl-dimethyl-ammoniumchlorid	Monoethanolamin	4,0
9	dto.	Diethanolamin	3,8
10	dto.	Triethanolamin	3,1
11	dto.	3-Amino-1-propanol	4,0
12	Di-C_{8-10}-alkyldimethyl-ammoniumchlorid (60%) / C_{12-16}-Alkyl-benzyl-di-methylammoniumchlorid (40%); Bardac® 205-M	Monoethanolamin	3,9
13	dto.	Diethanolamin	3,2
14	dto.	Triethanolamin	2,8
15	dto.	3-Amino-1-propanol	3,8
16	*N,N*-Bis(3-aminopropyl)-dodecylamin	Monoethanolamin	2,9
17	dto.	Diethanolamin	2,7
18	dto.	Triethanolamin	2,4
19	dto.	3-Amino-1-propanol	2,8

Zum Vergleich wurden alle in Tabelle 1 aufgeführten Verbindungen als Einzelsubstanzen in 0,5%iger Lösung getestet. Keine dieser Verbindungen wies eine ausgeprägte fungizide Wirkung auf (log_{10} Keimreduktion <2).

Beispiel 20

Es wurde eine Desinfektionsmittelformulierung (Konzentrat) hergestellt aus:

9,9% Didecyl-dimethylammoniumchlorid (70%ige Lösung)
8,0% Monoethanolamin
5,0% Genapol® T250 (Talgfettalkoholpolyglycolether, 25 mol Ethylenoxid)
5,0% Kaliumcarbonat (wasserfrei)
6,0% Ethylendiamintetraessigsäure-Tetranatriumsalz (Trilon® B; 40%ige Lösung)
Wasser ad 100%

Beispiel 21

Das in Beispiel 20 beschriebene Konzentrat wurde in 6%iger Verdünnung m Suspensionsversuch mit einer Einwirkzeit von 30, 60 und 120 Minuten auf Wirksamkeit gegen Poliovirus Typ 1 (Stamm Mahoney) geprüft.

Testmethode:

Die Prüfung erfolgte in Anlehnung an die „Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren" (*Bundesgesundheitsbl.* 1982, *25*, 397). Als Wachstumsmedium zur Vermehrung der Vero-Zellkulturen diente „Dulbecco's Modified Eagle's Medium", dem 10% fetales Kälberserum und 10 U/ml Penicillin sowie 10 µg/ml Streptomycin zugegeben war. Nach Inokulation der Gewebekultur mit Polio-Virus enthielt das Gewebekulturmedium nur noch 3% fetales Kälberserum. Nach fast vollständiger Ablösung Polio-infizierter Zellen wurde die Virussuspension durch Abzentrifugieren von Zellen und Zellbestandteilen (3000×g, 15 min) gereinigt. Da das Medium der Zellkultur 3% fetales Kälberserum enthielt, war auch bei der Desinfektionsmittelprüfung auch in den Testansätzen mit bidestilliertem Wasser eine geringe Eiweissbelastung vorhanden.
Zur Desinfektionsmittelprüfung wurde 1 Teil der Virussuspension mit 8 Teilen einer 7,5%igen Verdünnung des Desinfektionsmittelkonzentrats (entsprechend einer End-

konzentration von 6%) und jeweils 1 Teil Aqua bidest. oder 2%igem Serumalbumin oder fetalem Kälberserum gemischt und für 30, 60 und 120 min bei 20 °C inkubiert. Dann wurde die Wirkung des Desinfektionsmittels durch 100fache Verdünnung mit kaltem Medium, das kein fetales Kälberserum enthielt, gestoppt. Mit jeweils 1 ml dieser Verdünnung (entsprechend einer Verdünnung der Virussuspension auf 10^{-3}) und weiteren dekadischen Verdünnungsstufen wurden jeweils 2 Schälchen von Multiwellplatten mit 6 Vertiefungen (Becton Dickinson Labware, Lincoln Park, NJ, Typ Falcon™ 353046), die einen dichten Zellrasen von Vero-Zellen enthielten, inokuliert. Nach 1 h Adsorptionszeit bei Raumtemperatur wurde die überstehende Flüssigkeit abgesaugt. Dann wurde der Zellrasen der Schälchen mit 2 ml 2%iger, durch Kochen verflüssigter Agarose (Serva high EEO, Kat.-Nr. 11397), die mit doppelt konzentriertem Medium mit 5%igem fetalem Kälberserum im Verhältnis 1:1 gemischt und im Wasserbad auf 40 °C abgekühlt worden war, überschichtet. Nach Erstarren der Agarose bei Raumtemperatur wurden die Platten bei 37 °C im CO_2-Brutschrank für 2 Tage inkubiert.

Die Prüfung der Infektiosität der Virussuspension erfolgte im Plaque-Test. Dabei entspricht jedes Areal an zerstörten Zellen einer infektiösen Einheit des Poliovirus. Die Zahl der Plaques zeigt somit die Zahl der in einer bestimmten Verdünnung des Versuchsansatzes vorhandenen infektiösen Viruspartikel an. Die Plaques werden durch Anfärbung mit jeweils 1,0 ml einer Lösung von 0,1% Brilliant Blue R (Sigma, Kat.-Nr. B0149) in einer wässrigen Lösung mit 20% Methanol und 5% Essigsäure für 30 min sichtbar gemacht. Die ungefärbten Plaques heben sich anschliessend vom blau gefärbten Zellrasen deutlich ab. Aus jeweils zwei Ansätzen einer Verdünnung wurde ein Mittelwert der Plaquezahl errechnet.

Als „Viruskontrollen", in denen die Ausgangskonzentration des Virus bestimmt wurde, dienten Ansätze, in denen das Desinfektionsmittel durch das gleiche Volumen bidestilliertes Wasser ersetzt war. Die so ermittelte Viruskonzentration diente als Referenzwert für die Berechnung der virusinaktivierenden Wirkung der getesteten Desinfektionsmittel.

Als „Toxizitätskontrollen" zum Nachweis einer eventuellen Schädigung der Gewebekulturzellen durch das Desinfektionsmittel dienten Ansätze, in denen die Virussuspension durch das gleiche Volumen bidestilliertes Wasser ersetzt war. Diese Ansätze wurden im Verhältnis 1:100 und 1:1000 (entsprechend einer Verdünnung der Virussuspension von 10^{-3} bzw. 10^{-4} im Desinfektionsmittel-Testansatz) mit Medium ohne fetales Kälberserum verdünnt. Dann wurden sie wie die Ansätze zum Test der Desinfektionswirkung für 1 h der

2,0% Ethylendiamintetraessigsäure-Tetranatriumsalz (40%ige Lösung)

0,7% Ethylendiamintetraessigsäure

4,0% Butyldiglycol

Wasser ad 100%

Die Wirksamkeit wurde mit einer Verdünnung (1 Teil Konzentrat, 199 Teile Wasser) bei 20 °C und 15 min Kontaktzeit bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war >4,4.

Zusätzlich wurde die Wirksamkeit noch nach dem in CEN 1650 spezifizierten Verfahren bei einer Kontaktzeit von 15 min, einer Konzentration von 1,0%, einer Wasserhärte von 30 °fH und einer organischen Belastung von 0,3% Albumin bestimmt. Der dekadische Logarithmus der Keimzahlreduktion war >4,4.

Beispiele 4–19

Es wurden wässrige Lösungen aus 0,5% Alkanolamin (II) und 0,25% Amin bzw. quartäres Ammoniumsalz (Ia/Ib) hergestellt und nach dem in CEN 1275 spezifizierten Verfahren gestestet. Die Ergebnisse sind in der folgenden Tabelle 1 zusammengefasst.

Tabelle 1

Beispiel Nr.	Amin/Ammoniumsalz	Alkanolamin	log_{10} Keimreduktion
4	Dimethyl-dioctyl-ammoniumchlorid	Monoethanolamin	4,3
5	dto.	Diethanolamin	4,0
6	dto.	Triethanolamin	3,6
7	dto.	3-Amino-1-propanol	4,2
8	Didecyl-dimethyl-ammoniumchlorid	Monoethanolamin	4,0
9	dto.	Diethanolamin	3,8
10	dto.	Triethanolamin	3,1
11	dto.	3-Amino-1-propanol	4,0
12	Di-C_{8-10}-alkyldimethyl-ammoniumchlorid (60%) / C_{12-16}-Alkyl-benzyl-dimethylammoniumchlorid (40%); Bardac® 205-M	Monoethanolamin	3,9
13	dto.	Diethanolamin	3,2
14	dto.	Triethanolamin	2,8
15	dto.	3-Amino-1-propanol	3,8
16	*N,N*-Bis(3-aminopropyl)-dodecylamin	Monoethanolamin	2,9
17	dto.	Diethanolamin	2,7
18	dto.	Triethanolamin	2,4
19	dto.	3-Amino-1-propanol	2,8

Zum Vergleich wurden alle in Tabelle 1 aufgeführten Verbindungen als Einzelsubstanzen in 0,5%iger Lösung getestet. Keine dieser Verbindungen wies eine ausgeprägte fungizide Wirkung auf (log_{10} Keimreduktion <2).

Beispiel 20

Es wurde eine Desinfektionsmittelformulierung (Konzentrat) hergestellt aus:

9,9% Didecyl-dimethylammoniumchlorid (70%ige Lösung)
8,0% Monoethanolamin
5,0% Genapol® T250 (Talgfettalkoholpolyglycolether, 25 mol Ethylenoxid)
5,0% Kaliumcarbonat (wasserfrei)
6,0% Ethylendiamintetraessigsäure-Tetranatriumsalz (Trilon® B; 40%ige Lösung)
Wasser ad 100%

Beispiel 21

Das in Beispiel 20 beschriebene Konzentrat wurde in 6%iger Verdünnung m Suspensionsversuch mit einer Einwirkzeit von 30, 60 und 120 Minuten auf Wirksamkeit gegen Poliovirus Typ 1 (Stamm Mahoney) geprüft.

Testmethode:

Die Prüfung erfolgte in Anlehnung an die „Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren" (*Bundesgesundheitsbl.* 1982, *25*, 397). Als Wachstumsmedium zur Vermehrung der Vero-Zellkulturen diente „Dulbecco´s Modified Eagle´s Medium", dem 10% fetales Kälberserum und 10 U/ml Penicillin sowie 10 µg/ml Streptomycin zugegeben war. Nach Inokulation der Gewebekultur mit Polio-Virus enthielt das Gewebekulturmedium nur noch 3% fetales Kälberserum. Nach fast vollständiger Ablösung Polio-infizierter Zellen wurde die Virussuspension durch Abzentrifugieren von Zellen und Zellbestandteilen (3000×g, 15 min) gereinigt. Da das Medium der Zellkultur 3% fetales Kälberserum enthielt, war auch bei der Desinfektionsmittelprüfung auch in den Testansätzen mit bidestilliertem Wasser eine geringe Eiweissbelastung vorhanden.
Zur Desinfektionsmittelprüfung wurde 1 Teil der Virussuspension mit 8 Teilen einer 7,5%igen Verdünnung des Desinfektionsmittelkonzentrats (entsprechend einer End-

konzentration von 6%) und jeweils 1 Teil Aqua bidest. oder 2%igem Serumalbumin oder fetalem Kälberserum gemischt und für 30, 60 und 120 min bei 20 °C inkubiert. Dann wurde die Wirkung des Desinfektionsmittels durch 100fache Verdünnung mit kaltem Medium, das kein fetales Kälberserum enthielt, gestoppt. Mit jeweils 1 ml dieser Verdünnung (entsprechend einer Verdünnung der Virussuspension auf 10^{-3}) und weiteren dekadischen Verdünnungsstufen wurden jeweils 2 Schälchen von Multiwellplatten mit 6 Vertiefungen (Becton Dickinson Labware, Lincoln Park, NJ, Typ Falcon™ 353046), die einen dichten Zellrasen von Vero-Zellen enthielten, inokuliert. Nach 1 h Adsorptionszeit bei Raumtemperatur wurde die überstehende Flüssigkeit abgesaugt. Dann wurde der Zellrasen der Schälchen mit 2 ml 2%iger, durch Kochen verflüssigter Agarose (Serva high EEO, Kat.-Nr. 11397), die mit doppelt konzentriertem Medium mit 5%igem fetalem Kälberserum im Verhältnis 1:1 gemischt und im Wasserbad auf 40 °C abgekühlt worden war, überschichtet. Nach Erstarren der Agarose bei Raumtemperatur wurden die Platten bei 37 °C im CO_2-Brutschrank für 2 Tage inkubiert.

Die Prüfung der Infektiosität der Virussuspension erfolgte im Plaque-Test. Dabei entspricht jedes Areal an zerstörten Zellen einer infektiösen Einheit des Poliovirus. Die Zahl der Plaques zeigt somit die Zahl der in einer bestimmten Verdünnung des Versuchsansatzes vorhandenen infektiösen Viruspartikel an. Die Plaques werden durch Anfärbung mit jeweils 1,0 ml einer Lösung von 0,1% Brilliant Blue R (Sigma, Kat.-Nr. B0149) in einer wässrigen Lösung mit 20% Methanol und 5% Essigsäure für 30 min sichtbar gemacht. Die ungefärbten Plaques heben sich anschliessend vom blau gefärbten Zellrasen deutlich ab. Aus jeweils zwei Ansätzen einer Verdünnung wurde ein Mittelwert der Plaquezahl errechnet.

Als „Viruskontrollen", in denen die Ausgangskonzentration des Virus bestimmt wurde, dienten Ansätze, in denen das Desinfektionsmittel durch das gleiche Volumen bidestilliertes Wasser ersetzt war. Die so ermittelte Viruskonzentration diente als Referenzwert für die Berechnung der virusinaktivierenden Wirkung der getesteten Desinfektionsmittel.

Als „Toxizitätskontrollen" zum Nachweis einer eventuellen Schädigung der Gewebekulturzellen durch das Desinfektionsmittel dienten Ansätze, in denen die Virussuspension durch das gleiche Volumen bidestilliertes Wasser ersetzt war. Diese Ansätze wurden im Verhältnis 1:100 und 1:1000 (entsprechend einer Verdünnung der Virussuspension von 10^{-3} bzw. 10^{-4} im Desinfektionsmittel-Testansatz) mit Medium ohne fetales Kälberserum verdünnt. Dann wurden sie wie die Ansätze zum Test der Desinfektionswirkung für 1 h der

Gewebekultur zugegeben und dann abgezogen. Nach 2 Tagen Inkubation bei 37 °C wurde durch Anfärbung geprüft, ob der Zellrasen durch das Desinfektionsmittel geschädigt worden war.

Als Hinweis auf die Resistenz des Prüfvirus und zur Vergleichbarkeit mit anderen Untersuchungen wurde eine „Formaldehydkontrolle" mitgeführt. Dazu wurde 1 Teil der Virussuspension mit 4 Teilen phosphatgepufferter Kochsalzlösung (0,1 M; pH 7; „Dulbecco´s PBS") gemischt und die gleiche Menge einer 1,4 g Formaldehyd in 100 ml Lösung enthaltenden Formalinlösung (Endkonzentration: 0,7 g HCHO/100 ml) zugegeben. Nach 5, 15 und 60 min Einwirkzeit wurde die Wirksamkeit des Formaldehyds wie beim Desinfektionsmitteltest durch eine Verdünnung auf 1:100 gestoppt und die verbliebene Infektiosität des Poliovirus in weiteren dekadischen Verdünnungen im Plaque-Test bestimmt.

Ergebnisse:

Kontrollexperimente:

Die „Viruskontrolle" ergab im Ansatz mit bidestilliertem Wasser eine Viruskonzentration von $1{,}6 \cdot 10^8$ infektiösen Einheiten/ml, im Ansatz mit Serumalbumin $1{,}2 \cdot 10^8$ infektiösen Einheiten/ml und im Ansatz mit fetalem Kälberserum $1{,}0 \cdot 10^8$ infektiösen Einheiten/ml. Die „Toxizitätskontrolle" zeigte nach der Verdünnung des Prüfansatzes auf 1:100 (entsprechend einer Verdünnung der Virussuspension von 10^{-3}) eine leichte Schädigung des Zellrasens. Bei einer Verdünnung von 1:1000 war keine Toxizität mehr erkennbar. Somit kann unter den Testbedingungen eine Abnahme der Viruskonzentration unter Desinfektionsmitteleinwirkung bis zu einer Viruskonzentration von $5 \cdot 10^3$ infektiöse Einheiten/ml in der Virussuspension (in beiden Schälchen der Verdünnung 10^{-4} ist dann kein Plaque mehr sichtbar) verfolgt werden und bei einer Ausgangskonzentration von mindestens 10^8 infektiösen Viruspartikeln/ml ist eine Abnahme der Viruskonzentration über mindestens 4,5 Zehnerpotenzen beobachtbar. Da die Prüfrichtlinie zum Nachweis der Wirksamkeit eines Desinfektionsmittels nur eine Abnahme der Viruskonzentration um wenigstens 4 Zehnerpotenzen verlangt, ist die Erfüllung dieser Bedingung mit dem gewählten Versuchsansatz überprüfbar.

Im Ansatz mit 0,7%igem Formaldehyd wurde nach 5 min Einwirkzeit eine Viruskonzentration von $1{,}05 \cdot 10^6$/ml, nach 15 min $1 \cdot 10^3$/ml und nach 60 min $\leq 5 \cdot 10^2$/ml gemessen. Dies sind erwartete Werte, die die Resultate früherer Versuche bestätigen: 0,7%iges Formalin

ist normalerweise in der Lage, die Konzentration von Poliovirus innerhalb von 30 min um mehr als 4 Zehnerpotenzen zu reduzieren.

Wirksamkeit der Desinfektionsmittel gegen Poliovirus:
Nach 30, 60 und 120 min Einwirkung von 6%iger Verdünnung der Desinfektionsmittelzusammensetzung aus Beispiel 20 wurde im Ansatz mit fetalem Kälberserum bei der Virusverdünnung 10^{-4} jeweils in beiden Testschälchen kein Plaque mehr beobachtet. Somit ist nach der Desinfektionsmittelbehandlung eine Viruskonzentration von $\leq 5 \cdot 10^3$ infektiöse Einheiten/ml vorhanden. Dieses Ergebnis wurde sowohl bei geringer (Ansatz mit Aqua bidest.) als auch mittlerer (Ansatz mit 2%igem Serumalbumin) und hoher (Ansatz mit fetalem Kälberserum) Eiweissbelastung gefunden. Es war damit im Vergleich zur Kontrollbestimmung ohne Desinfektionsmittel zu einer Abnahme der Viruskonzentration um mindestens 4,5 $\log_{10}$-Stufen bzw. Zehnerpotenzen gekommen.
Damit ist die Wirksamkeitsvoraussetzung für die Registrierung als Instrumentendesinfektionsmittel in der Bundesrepublik Deutschland erfüllt.

Beispiel 22

Wirksamkeit gegen ECBO-Viren:
Das in Beispiel 20 beschriebene Konzentrat wurde in Anlehnung an die Richtlinie des (deutschen) Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten e.V. zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren (*Bundesgesundheitsbl.* 1982, *25*, 397–398; Kommentar: *Bundesgesundheitsbl.* 1983, *26*, 413–414) im quantitativen Suspensionsversuch auf seine viruziden Eigenschaften gegenüber dem ECBO-Virus Stamm LCR-4 getestet. Untersucht wurden Verdünnungen von 1,0%, 3,0% und 5,0% des Konzentrats in bidestilliertem Wasser bei Einwirkzeiten von 15, 30, 60 und 120 min.
Die Prüftemperatur betrug 20±1 °C, als Eiweissbelastung wurde fetales Kälberserum (FKS) oder Serumalbumin (bovine serum albumin, BSA) verwendet.
Zur Herstellung der Virussuspension wurden fetale Kälberlungenzellen (FKL 107) in Roux-Flaschen mit Minimum Essential Medium (MEM, Eagle) mit ca. 10 PBE (Plaque bildende Einheiten) des Virus (erhalten von Dr. W. Herbst, Institut für Hygiene und

Infektionskrankheiten der Tiere an der Justus-Liebig-Universität in Giessen) pro Zelle infiziert und nach Erscheinen des cytopathischen Effekts (ca. 12 h) einem dreifachen Einfrier-/Auftauvorgang unterworfen. Es folgte eine Zentrifugation bei 770 × g für 10 min, welche die Virussuspension als Überstand lieferte.

Zur Herstellung der Inaktivierungsansätze wurden 8 Volumenteile des Desinfektionsmittels in der 1,25fachen gewünschten Konzentration mit Virussuspension und bidestilliertem Wasser (je 1 Volumenteil) vermischt. Bei den Versuchen mit Eiweissbelastung wurde anstelle des bidestillierten Wassers ein Volumenteil FKS (Flow Laboratories) bzw. 2%ige BSA-Lösung (Behringwerke AG) verwendet.

Die Durchführung der Inaktivierungsversuche erfolgte in geschlossenen Glasröhrchen. Nach den entsprechenden Zeiten wurde Proben entnommen, um die Restinfektiosität zu bestimmen.

Die Bestimmung der Infektiosität erfolgte mit Hilfe der Endverdünnungstitration im Mikroverfahren. Dazu wurden die Proben unmittelbar nach ihrer Entnahme mit Minimum Essential Medium (MEM) verdünnt, wobei als Verdünnungsfaktoren ganzzahlige Potenzen von 10 gewählt wurden. Jeweils 100 µl einer verdünnten Probe wurden in 8 Näpfe einer sterilen Polystyrolplatte mit flachem Boden übergeführt. Dann wurden jeweils 100 µl einer frisch trypsinierten Suspension von KOP-R-Zellen (Ösopharynxgewebe vom Rind, erhalten von Dr. R. Riebe, Bundesforschungsanstalt für Viruskrankheiten der Tiere auf der Insel Riems, Katalog-Nr. RIE 244) zugegeben. Diese Suspension war so eingestellt, dass sich in jedem Napf ca. 10–15 × 10^3 Zellen befanden. Danach wurden die Proben bei 37 °C im CO_2-Brutschrank (5 Vol% CO_2) inkubiert. Nach 5 bis 7 Tagen wurde nach der Methode von Spearmann-Kärber die infektiöse Dosis (ID_{50}/ml) bestimmt.

Die Beurteilung der viruziden Wirkung erfolgte durch Berechnung des Titerabfalls gegenüber den jeweils parallel (nach der längsten Einwirkzeit) durchgeführten Kontrolltitrationen. Die Differenz wurde als $\Delta\log_{10}$ ID_{50} angegeben.

Für die Bestimmung der Cytotoxizität des Desinfektionsmittels wurden 2 Volumenteile PBS (phosphate buffered saline) mit 8 Volumenteilen der Desinfektionsmittel-Verdünnung (1,25fache Konzentration) gemischt, entsprechend verdünnt und auf die Zellkulturen gebracht. Die Angabe der cytotoxischen Dosis erfolgte als $\log_{10}$ CD_{50}/ml (in Analogie zum ID_{50}-Wert).

Die Ergebnisse der Inaktivierungsversuche sind in der folgenden Tabelle 2 zusammengefasst, diejenigen der Cytotoxizitätsbestimmung in Tabelle 3.

Tabelle 2

Konzentration	Virusgehalt (Kontrolle) (log_{10} ID_{50}/ml)	Eiweiss-belastung	Abnahme des Infektionstiters (Δlog_{10} ID_{50}) nach			
			15 min	30 min	60 min	120 min
1,0%	6,85	—	1,25	1,25	1,64	n.d.
1,0%	7,05	0,2% BSA	1,00	1,25	1,34	n.d.
1,0%	7,15	10,0% FKS	1,00	1,15	1,25	n.d.
3,0%	7,65	—	≥4,15	≥4,15	≥4,15	≥4,15
3,0%	7,75	0,2% BSA	3,45	≥4,25	≥4,25	≥4,25
3,0%	7,65	10,0% FKS	2,17	3,45	≥4,15	≥4,15
5,0%	7,65	—	≥4,15	≥4,15	≥4,15	≥4,15
5,0%	7,75	0,2% BSA	4,05	≥4,25	≥4,25	≥4,25
5,0%	7,65	10,0% FKS	3,15	≥4,15	≥4,15	≥4,15

n.d. = nicht durchgeführt

Tabelle 3

Konzentration	Verdünnungsstufe				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1,0%	+	–	–	–	–
3,0%	+	+	–	–	–
5,0%	+	+	–	–	–

Die Ergebnisse zeigen, dass die getestete Zusammensetzung (Konzentrat) bei einer Anwendungskonzentration von 3,0% nach 60 min Einwirkzeit und bei 5,0% nach 30 min die in der Richtlinie definierte Wirksamkeit (Δlog_{10} $ID_{50} \geq 4,0$; entsprechend einer Inaktivierung von ≥99,99%) gegen ECBO-Viren aufweist.

Beispiel 23

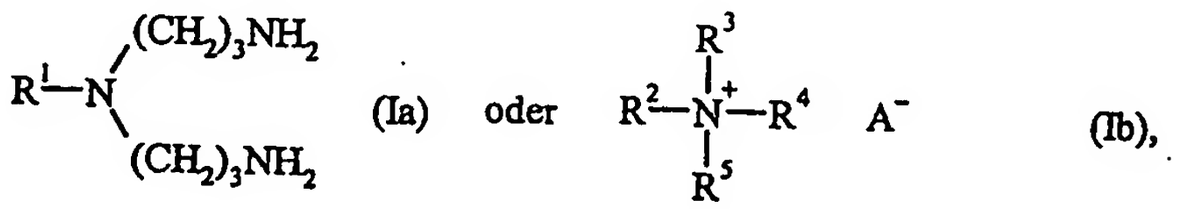
Wirksamkeit gegen Canine Parvovirus:

Verdünnungen des in Beispiel 20 beschriebenen Konzentrats wurden auf ihre Wirksamkeit gegen Canine Parvovirus Typ 2 (bezogen von Dr. Parrish, Cornell University) in NLFK-Zellen (Norden Lab Feline Kidney) bei 22 °C und 10 min Einwirkzeit getestet. Verdünnungen im Verhältnis 1:35 in entsalztem oder hartem (400 ppm AOAC hard water) Wasser mit 5% organischer Belastung (fetales Kälberserum) zeigten hinreichende viruzide Wirksamkeit.

Patentansprüche

1. Verwendung einer Desinfektionsmittelzusammensetzung, enthaltend

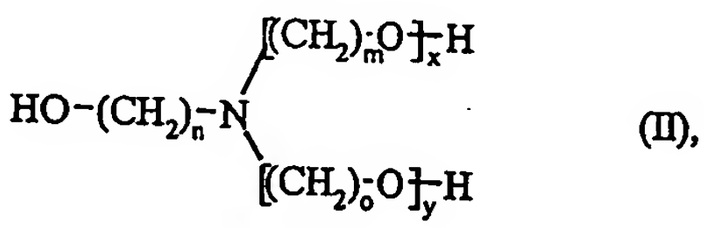
   a) ein Amin und/oder quartäres Ammoniumsalz der allgemeinen Formel



(Ia) oder (Ib),

   worin R^1 C_{6-18}-Alkyl,
   R^2 Benzyl oder C_{6-18}-Alkyl,
   R^3 C_{1-18}-Alkyl oder $-[(CH_2)_z-O]_nR^6$ mit $n = 1-20$,
   R^4 und R^5 unabhängig voneinander C_{1-4}-Alkyl,
   R^6 Wasserstoff oder gegebenenfalls substituiertes Phenyl
   und A^- ein einwertiges Anion oder ein Äquivalent eines mehrwertigen Anions einer anorganischen oder organischen Säure bedeutet; und

   b) wenigstens ein Alkanolamin der allgemeinen Formel



(II),

   worin n und, soweit vorhanden, m und o unabhängig voneinander den Wert 2 oder 3 und x und y unabhängig voneinander den Wert 0 oder 1 haben, oder ein entsprechendes Salz;

   im Massenverhältnis I:II von 20:1 bis 1:20
   als viruzides Mittel.

2. Verwendung nach Anspruch 1, dadurch gekennzeichnet, dass das Amin bzw. quartäre Ammoniumsalz ausgewählt ist aus der Gruppe bestehend aus *N,N*-Bis-(3-aminopropyl)dodecylamin, *N,N*-Bis(3-aminopropyl)octylamin, Didecyl-dimethylammoniumsalzen, Dioctyl-dimethylammoniumsalzen, Octyl-decyl-dimethylammoniumsalzen,

Kokosalkyl-dimethyl-benzylammoniumsalzen und Benzyl-dimethyl-oxethyl-ammoniumsalzen sowie Gemischen dieser Verbindungen.

3. Verwendung nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass das Alkanolamin (II) ausgewählt ist aus der Gruppe bestehend aus Monoethanolamin, Diethanolamin, Triethanolamin und 3-Amino-1-propanol.

4. Verwendung nach einem der Ansprüche 1 bis 3, dadurch gekennzeichnet, dass das Massenverhältnis I:II zwischen 1:5 und 5:1 liegt.

5. Verwendung nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass die Desinfektionsmittelzusammensetzung Wasser als Lösungsmittel enthält.

6. Verwendung nach einem der Ansprüche 1 bis 5, dadurch gekennzeichnet, dass die Desinfektionsmittelzusammensetzung zusätzlich einen oder mehrere Hilfsstoffe aus der Gruppe bestehend aus organischen Lösungsmitteln, Tensiden, Komplexbildnern, Duftstoffen und Farbstoffen enthält.

7. Verwendung nach einem der Ansprüche 1 bis 6 in der Flächen- und Instrumenten-desinfektion.

8. Verwendung nach einem der Ansprüche 1 bis 6 in der Wäschedesinfektion.

9. Verwendung nach einem der Ansprüche 1 bis 6 in der Händedesinfektion.

10. Verwendung nach einem der Ansprüche 1 bis 6 in chemischen Toiletten.

11. Verwendung nach einem der Ansprüche 1 bis 10 gegen Parvo- oder Picornaviren, insbesondere gegen Polioviren.

Hon. Commissioner of Patents and Trademarks.
P.O. Box 1450
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10/501395

Sir:

By placing your receiving date stamp hereon and mailing, kindly acknowledge receipt of:

A Transmittal letter to the US Designated Office with fee calculation worksheet (in duplicate), an executed oath of the inventors (in German with English-language translation), a copy of the published international application as followed and a copy of the following forms: PCT/ISA/210, PCT/IPEA/409, PCT/IB/332, PCT/IB/301, and a check in the amount of nine hundred twenty dollars ($920.00) for the national stage application of:

Applicants: Florian LICHTENBERG et al.
Serial No.: Unknown (based on PCT/EP03/00378)
Filed: 07/15/2004
For: VIRUCIDAL COMPOSITIONS

Hand-Carried on July 15 2004
LP-1940

FORM PTO-1390 (REV. 10-2003)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: LP-1940

U.S. APPLICATION NO. (If known, see 37 CFR 1.5):

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP03/00378	01/16/2003	01/18/2002

TITLE OF INVENTION: VIRUCIDAL DISINFECTANT

APPLICANT(S) FOR DO/EO/US: Florian LICHTENBERG, Michael LÜTZELER, Volker RANFT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [XX] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [XX] The US has been elected (Article 31).
5. [XX] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [XX] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [XX] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A preliminary amendment.
14. [ ] An Application Data Sheet under 37 CFR 1.76.
15. [ ] A substitute specification.
16. [ ] A power of attorney and/or change of address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13*ter*.2 and 37 CFR 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [XX] Other items or information: a copy of the published international application, a translation of the German-language declaration, a copy of the following forms: PCT/ISA/210, PCT/IPEA/409, PCT/IB/332, PCT/IB/301

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/EP03/00378	LP-1940

21. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1080.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$920.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$770.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$730.00**		
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Surcharge of **$130.00** for furnishing the oath or declaration later than 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total claims	11 - 20 =	0	x **$18.00**	$ 0	
Independent claims	1 - 3 =	0	x **$86.00**	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$290.00**	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 920.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$	
SUBTOTAL =				$ 920.00	
Processing fee of **$130.00** for furnishing the English translation later than 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ 920.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 920.00	
				Amount to be refunded:	$
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NOTE: Where an appropriate time limit under 37 CFR 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

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(54) VIRUCIDAL DISINFECTANT

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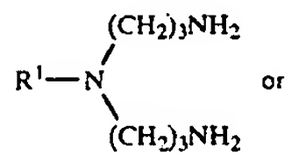
(57) ABSTRACT

Process for utilizing the disinfectant composition as a virucidal agent. The disinfectant composition includes:

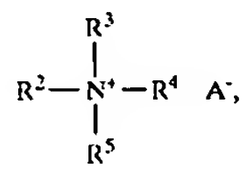
(a) an amine and/or quaternary ammonium salt of the general formula:

(Ia)



or

(Ib)



where R^1 is C_{6-8}-alkyl,

R^2 is benzyl or C_{6-18}-alkyl,

R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n=1-20,

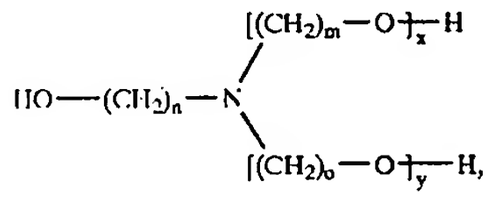
R^4 and R^5 independently of one another are C_{1-4}-alkyl,

R^6 is hydrogen or unsubstituted or substituted phenyl, and

A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

(b) at least one alkanolamine of the general formula:

(II)



where n and, if present, m and o independently of one another have the value 2 or 3, and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio of (a) to ( )b) of 20:1 to 1:20.

VIRUCIDAL DISINFECTANT

[0001] The invention relates to the use of synergistic disinfectant compositions based on amines and/or quaternary ammonium salts as virucidal agents, in particular against polioviruses.

[0002] Numerous disinfectant and preservative compositions based on amines and/or quaternary ammonium salts are known. However, in general, in particular at relatively high dilution, these exhibit an unsatisfactory activity towards fungi, for example *Aspergillus niger* and viruses (in particular towards highly resistant viruses, for example polioviruses).

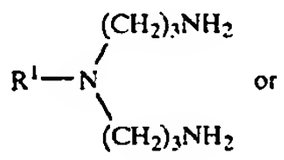
[0003] It was therefore an object of the present invention to provide disinfectant compositions based on amines and/or quaternary ammonium salts which exhibit good activity towards fungi and in particular towards viruses even at high dilution.

[0004] This object is achieved according to the invention by the use according to Claim 1.

[0005] The earlier application PCT/EP 01/10754 (published as WO 02/23990 A1) describes disinfectant compositions based on amines and/or quatemary ammonium salts and alkanolamines and their fungicidal properties. It has now surprisingly been found that such disinfectant compositions also display pronounced virucidal properties and, in particular, also good activity towards highly resistant viruses such as polioviruses. They are likewise active against other picornaviruses, for example ECHO viruses or corresponding animal pathogen viruses such as ECBO viruses, and also against parvoviruses, for example canine parvovirus.

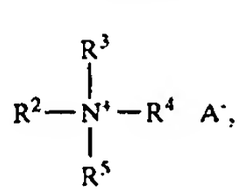
[0006] The compositions comprise amines and/or quaternary ammonium salts of the general formula

(Ia)



or

(Ib)



[0007] where R^1 is C_{6-18}-alkyl

[0008] R^2 is benzyl or C_{6-18}-alkyl

[0009] R^3 is C_{1-18}-alkyl or $—[(CH_2)_2—O]_nR^6$ where n=1-20

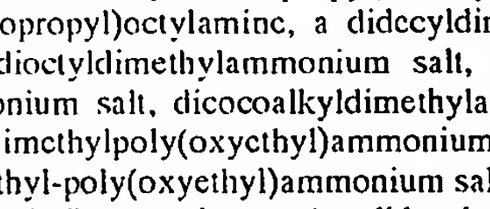
[0010] R^4 and R^5 independently of one another are C_{1-4}-alkyl

[0011] R^6 is hydrogen or unsubstituted or substituted phenyl

[0012] and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid;

[0013] and at least one alkanolamine of the general formula

(II)



[0014] where n and, if present, m and o independently of one another have the value 2 or 3

[0015] and x and y independently of one another have the-value 0 or 1, or a corresponding salt; in the mass ratio I:II of 20:1 to 1:20.

[0016] Alkyi, here and hereinafter, is taken to mean in each case unbranched or branched alkyl groups of the specified number of carbons, but preferably unbranched alkyl groups, and particularly preferably those having an even number of carbon atoms. In particular, this is also taken to mean the homologue mixtures derived from natural raw materials, for example "cocoalkyl".

[0017] Substituted phenyl is taken to mean, in particular, phenyl groups substituted with one or more C_{1-18}-alkyl groups and/or chlorine atoms. Suitable anions A^- are in principle all inorganic or organic anions, in particular halide, for example chloride or bromide, or anions of low carboxylic acids, for example acetate, propionate or lactate.

[0018] The amine or quaternary ammonium salt (Ia/Ib) is preferably N,N-bis(3-aminopropyl)dodecylamine, N,N-bis(3-aminopropyl)octylamine, a didecyldimethylammonium salt, dioctyldimethylammonium salt, octyldecyldimethylammonium salt, dicocoalkyldimethylammonium salt, cocoalkyldimethylpoly(oxyethyl)ammonium salt, dicocoalkylmethyl-poly(oxyethyl)ammonium salt, decyldimethylpoly(oxyethyl)ammonium salt, didecylmethylpoly(oxyethyl)ammonium salt, octyidimethylpoly(oxyethyl)ammonium salt, dioctylmethylpoly(oxyethyl)ammonium salt, coco-alkyt-dimethylbenzylammonium salt, benzyldodecyidimethylammonium salt or benzyldimethylpoly(oxyethyl)ammonium salt or a mixture of two or more of these compounds. Particularly good results were achieved with didecyldimethylammonium salts.

[0019] Suitable alkanolamines (II) are in principle all ethanolamines and propanolamines, in particular monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol. Obviously, using mixtures of the said compounds is also within the scope of the invention. Particularly good results have been obtained using the compounds having a primary amino group, that is to say using monoethanolamine and 3-amino-1-propanol.

[0020] The mass ratio of amine (Ia) or quatemary ammonium salt (Ib) to alkanolamine (II) is preferably in the range from 1:5 to 5:1.

[0021] The disinfectant compositions used according to the invention preferably comprise water as solvent, if appropriate in combination with an organic solvent.

[0022] Preferably, the disinfectant compositions used according to the invention further comprise one or more aids

selected from the group consisting of organic solvents, surfactants, complexing agents fragrances and colorants.

[0023] A preferred use of the disinfectant compositions is surface disinfection and instrument disinfection.

[0024] Further preferred fields of use are laundry disinfection and hand disinfection.

[0025] A further preferred use of the disinfectant compositions is the use in chemical toilets, for example on board aircraft and vehicles.

[0026] The examples below illustrate the implementation of the invention, and should not be taken to be a restriction to the embodiments described. All quantities given, where not otherwise specified, are in % by mass. The test microorganism used in each case was *Aspergillus niger* ATCC 16404. The effectiveness was determined, unless otherwise specified, using the method specified in CEN 1275.

EXAMPLE 1

[0027] A disinfecting cleaner formulation (concentrate) was prepared from:

[0028] 5.0% didecyldimethylammonium chloride (50% strength solution)

[0029] 2.0% N,N-bis(3-aminopropyl)dodecylamine

[0030] 5.0% monoethanolamine

[0031] 5.0% Genapol® 250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)

[0032] 0.5% sodium metasilicate

[0033] 0.5% sodium carbonate

[0034] 2.0% methylglycinediacetic acid trisodium salt (Trilon® M; 40% strength solution)

[0035] water to 100%

[0036] The effectiveness was determined using a dilution (1 part of concentrate, 99 parts of water) at 20° C. and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.1.

COMPARATIVE EXAMPLE 1

[0037] The procedure of Example 1 was followed, but with the difference that the monoethanolamine was replaced by the same amount of water. Under the same test conditions, the formulation was virtually inactive.

EXAMPLE 2

[0038] A disinfectant formulation (concentrate) was prepared from:

[0039] 4.9% N,N-bis(3-aminopropyl)dodecylamine

[0040] 4.0% monoethanolamine

[0041] 2.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)

[0042] 5.0% Hostapur® SAS 30 (C_{13-17} secondary n-alkanesulfonic acid, sodium salt)

[0043] 2.0% ethylenediaminetetraacetic acid tetrasodium salt (40% strength solution)

[0044] 0.7% ethylenediaminetetraacetic acid

[0045] water to 100%

[0046] The effectiveness was determined using a dilution (1 part of concentrate, 199 parts of water) at 20° C. and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was 4.3.

EXAMPLE 3

[0047] A disinfectant formulation (concentrate) was prepared from:

[0048] 4.2% N,N-bis(3-aminopropyl)dodecylamine

[0049] 2.0% didecylmethylpoly(oxyethyl)ammonium propionate (BARDAP 26)

[0050] 4.0% monoethanolamine

[0051] 2.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)

[0052] 5.0% Hostapur® SAS 30 (C_{13-17} secondary n-alkanesulfonic acid, sodium salt)

[0053] 2.0% ethylenediaminetetraacetic acid tetrasodium salt (40% strength solution)

[0054] 0.7% ethylenediaminetetraacetic acid

[0055] 4.0% butyl diglycol

[0056] water to 100%

[0057] The effectiveness was determined using a dilution (1 part of concentrate, 199 parts of water) at 20° C. and with a contact time of 15 min. The logarithm to base ten of the reduction in microorganism count was >4.4. In addition, the effectiveness was also determined using the method specified in CEN 1650 with a contact time of 15 min, a concentration of 1.0%, a water hardness of 30°fH and an organic load of 0.3% albumin. The logarithm to base ten of the reduction in microorganism count was >4.4.

EXAMPLES 4-19

[0058] Aqueous solutions were prepared from 0.5% alkanolamine (II) and 0.25% of amine or quaternary ammonium salt (Ia/Ib) and tested using the method specified in CEN 1275. The results are summarized in Table 1 below.

TABLE I

Example No.	Amine/ammonium salt	Alkanolamine	$\log_{10}$ microbial reduction
4	dimethyldioctylammonium chloride	monoethanolamine	4.3
5	dimethyldioctylammonium chloride	diethanolamine	4.0
6	dimethyldioctylammonium chloride	triethanolamine	3.6
7	dimethyldioctylammonium chloride	3-amino-1-propanol	4.2
8	didecyldimethylammonium chloride	monoethanolamine	4.0
9	didecyldimethylammonium chloride	diethanolamine	3.8
10	didecyldimethylammonium chloride	triethanolamine	3.1
11	didecyldimethylammonium chloride	3-amino-1-propanol	4.0

TABLE 1-continued

Example No.	Amine/ammonium salt	Alkanolamine	log_{10} microbial reduction
12	di-C_{8-10}-alkyldimethyl-ammonium chloride (60%)/C_{12-16}-alkyl-benzyldimethylammonium chloride (40%); Bardac ® 205-M	monoethanolamine	3.9
13	di-C_{8-10}-alkyldimethyl-ammonium chloride (60%)/C_{12-16}-alkyl-benzyldimethylammonium chloride (40%); Bardac ® 205-M	diethanolamine	3.2
14	di-C_{8-10}-alkyldimethyl-ammonium chloride (60%)/C_{12-16}-alkyl-benzyldimethylammonium chloride (40%); Bardac ® 205-M	triethanolamine	2.8
15	di-C_{8-10}-alkyldimethyl-ammonium chloride (60%)/C_{12-16}-alkyl-benzyldimethylammonium chloride (40%); Bardac ® 205-M	3-amino-1-propanol	3.8
16	N,N-bis(3-amino-propyl)dodecylamine	monoethanolamine	2.9
17	N,N-bis(3-amino-propyl)dodecylamine	diethanolamine	2.7
18	N,N-bis(3-amino-propyl)dodecylamine	triethanolamine	2.4
19	N,N-bis(3-amino-propyl)dodecylamine	3-amino-1-propanol	2.8

[0059] For comparison, all compounds listed in Table 1 were tested as individual substances in 0.5% strength solution. None of theses compounds exhibited pronounced fungicidal activity (log_{10} microbial reduction <2).

EXAMPLE 20

[0060] A disinfectant formulation (concentrate) was produced from:

[0061] 9.9% didecyidimethylammonium chloride (70% strength solution)

[0062] 8.0% monoethanolamine

[0063] 5.0% Genapol® T250 (tallow fatty alcohol polyglycol ether, 25 mol of ethylene oxide)

[0064] 5.0% potassium carbonate (anhydrous)

[0065] 6.0% ethylenediaminetetraacetic acid tetrasodium salt (Trilon®B; 40% strength solution)

[0066] water to 100%

EXAMPLE 21

[0067] The concentrate described in Example 20 was tested in 6% strength dilution in the suspension test using an exposure time of 30, 60 and 120 minutes for effectiveness against poliovirus type 1 (Mahoney strain).

[0068] Test Method:

[0069] The test was performed in accordance with the "Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren"[Guideline of the German Federal Health Agency and the German Association for Controlling Viral Diseases for testing chemical disinfectants for effectiveness against viruses] (*Bundesgesundheitsbl.* 1982, 25, 397). The growth medium for the Vero cell cultures was "Dulbecco's Modified Eagle's Medium", to which 10% foetal calf serum and 10 U/ml of penicillin and also 10 μg/ml of streptomycin had been added. After the tissue culture was inoculated with poliovirus the tissue culture medium only contained 3% foetal calf serum. After virtually complete detachment of polio-infected cells, the virus suspension was purified by centrifuging of cells and cell constituents (3000×g, 15 min). Since the cell culture medium contained 3% foetal calf serum, in the disinfectant test also, a small protein load was also present in the test batches using twice-distilled water.

[0070] For the disinfectant test, 1 part of virus suspension was mixed with 8 parts of a 7.5% strength dilution of the disinfection concentrate (corresponding to a final concentration of 6%) and in each case 1 part of twice-distilled water or 2% strength serum albumin or foetal calf serum and was incubated for 30, 60 and 120 min at 20° C. The activity of the disinfectant was then stopped by 100-fold dilution with cold medium containing no foetal calf s serum. In each case 2 wells of multiwell plates containing 6 recesses (Becton Dickinson Labware, Lincoln Park, N.J., Type Falcon™353046) which contained a dense lawn of Vero cells, were inoculated with 1 ml in each case of this dilution (corresponding to a dilution of the virus suspension to 10-3) and further serial 10-fold dilutions. After 1 h of adsorption time at room temperature, the supernatant liquid was drawn off. The cell lawns of the wells were then coated with 2 ml of 2% strength agarose (Serva high EEO, Cat. No. 11397) liquefied by boiling, which had been mixed with twice-concentrated medium containing 5% strength foetal calf serum in a ratio of 1:1, and had been cooled to 40° C. in a waterbath. After solidification of the agarose at room temperature, the plates were incubated for 2 days at 37° C. in a CO_2 incubation cabinet.

[0071] The infectivity of the virus suspension was tested in the plaque test. In this test each area of destroyed cells corresponds to one infectious unit of poliovirus. The number of plaques thus indicates the number of infectious virus particles present in a defined dilution of the test batch. The plaques are visualized by staining 1.0 ml in each case of a solution of 0.1% Brilliant Blue R (Sigma, Cat. No. B0149) for 30 min in an aqueous solution containing 20% methanol and 5% acetic acid. The unstained plaques are then clearly differentiated from blue-coloured cell lawns. A mean plaque count is calculated from two batches in each case of a dilution.

[0072] "Virus controls", in which the starting concentration of the virus was determined, were batches in which the disinfectant had been replaced by the same volume of twice-distilled water. The virus concentration thus determined served as reference for calculating the virus-inactivating action of the disinfectant tested. "Toxicity controls" for detecting any damage of the tissue culture cell by the disinfectant were batches in which the virus suspension had been replaced by the same volume of twice-distilled water. These batches were diluted in a ratio of 1:100 and 1:1000 (equivalent to a dilution of the virus suspension of 10^{-3} and

10^{-4} in the disinfectant test batch) with medium without foetal calf serum. Then they were added to the tissue culture, as with the batches for testing the disinfectant action, for 1 h and then drawn off. After incubation for 2 days at 37° C., staining was used to test whether the cell lawn had been damaged by the disinfectant.

[0073] As an indication of the resistance of the test virus and for comparability with other studies, a "formaldehyde control" was carried out. For this, 1 part of the virus suspension was mixed with 4 parts of phosphate-buffered saline (0.1 M; pH 7; "Dulbecco's PBS") and the entire volume was added to a formalin solution containing 1.4 g of formaldehyde in 100 ml of solution (final concentration: 0.79 of HCHO/100 ml). After 5, 15 and 60 min of exposure time, the action of formaldehyde was stopped, as with the disinfectant test, by diluting to 1:100 and the remaining infectivity of the poliovirus was determined in the plaque test in further serial ten-fold dilutions.

[0074] Results:

[0075] Control Experiments:

[0076] The "virus control", in the batch with twice-distilled water, gave a virus concentration of $1.6 \cdot 10^8$ infectious units/ml, in the batch containing serum albumin, $1.2 \cdot 10^8$ infectious units/ml, and in the batch containing foetal calf serum $1.0 \cdot 10^8$ infectious units/ml. The "toxicity control", after dilution of the test batch to 1:100 (equivalent to a dilution of the virus suspension of 10^{-3}) showed slight damage of the cell lawn. At a dilution of 1:1000, toxicity was no longer observable. Thus under the test conditions, a decrease in virus concentration under the action of disinfectant can be followed to a virus concentration of $5 \cdot 10^3$ infectious units/ml in the virus suspension (in both wells of the dilution 10^{-4}, plaque is then no longer visible) and at a starting concentration of at least 10^8 infectious virus particles/ml, a decrease in virus concentration over at least 4.5 powers of ten is observable. Since the test guideline for detecting the effectiveness of a disinfectant only requires a decrease in virus concentration by at least 4 powers of ten, compliance with this condition can be detected using the experimental batch chosen. In the batch containing 0.7% strength formaldehyde, after an exposure time of 5 min, a virus concentration of $1.05 \cdot 10^6$/ml was measured, after 15 min $1 \cdot 10^3$/ml, and after 60 min $\leqq 5 \cdot 10^2$/ml. These are expected values which confirm the results of earlier experiments: 0.7% strength formalin is usually able to reduce the concentration of poliovirus by more than 4 powers of ten within 30 min.

[0077] Effectiveness of the Disinfectants Against Poliovirus:

[0078] After 30, 60 and 120 min exposure times of 6% strength dilution of the disinfectant composition from Example 20, in the batch containing foetal calf serum at the virus dilution 10^4, in each of the two test wells plaque was no longer observed. Thus after the disinfectant treatment, a virus concentration of $\leqq 5 \cdot 10^3$ infectious units/ml was present. This result was found not only with low protein load (batch with twice-distilled water), but also with medium (batch containing 2% strength serum albumin) and high protein load (batch containing foetal calf serum). Thus, compared with the control determination without disinfectant, there was a decrease in virus concentration by at least 4.5 $\log_{10}$ or powers of ten.

[0079] Thus the condition for effectiveness for registration as instrument disinfectant in the Federal Republic of Germany is fulfilled.

EXAMPLE 22

[0080] Effectiveness Against ECBO Viruses:

[0081] The concentrate described in Example 20 was tested in accordance with the guideline of the (German) Federal Health Agency and the Deutsche Vereinigung zur Bekäimpfung der Viruskrankheiten e.V. for testing chemical disinfectants for effectiveness against viruses (*Bundesgesundheitsbl.* 1982, 25, 397-398; comment: *Bundesgesundheitsbl.* 1983, 26, 413414) in a quantitative suspension test for its virucidal properties against the ECBO virus strain LCR4. Tests were made of dilutions of 1.0%, 3.0% and 5.0% of the concentrate in twice-distilled water with exposure times of 15, 30, 60 and 120 min.

[0082] The test temperature was 20±1° C., and the protein load used was foetal calf serum (FCS) or serum albumin (bovine serum albumin, BSA).

[0083] To prepare the virus suspension, foetal calf lung cells (FCL 107) in Roux flasks containing minimum essential medium (MEM, Eagle) were infected with approximately 10 PFU (plaque forming units) of the virus (obtained by Dr W. Herbst, Institute for Hygiene and Infectious Animal Diseases at the Justus-Liebig University in Giessen) per cell and after appearance of the cytopathic effect (approximately 12 h), was subjected to three-fold freezing/thawing operation. There followed centrifugation at 770×g for 10 min which provided the virus suspension as supernatant.

[0084] To prepare the inactivation batches, 8 parts by volume of the disinfectant in the desired 1.25-fold concentration were mixed with virus suspension and twice-distilled water (1 part by volume each). In the experiments with protein load, instead of the twice-distilled water, one part by volume of FCS (Flow Laboratories) or 2% strength BSA solution (Behringwerke A G) were used.

[0085] The inactivation experiments were carried out in closed glass tubes. After the appropriate times, samples were withdrawn to determine the remaining infectivity.

[0086] The infectivity was determined using end-dilution titration in the micro method. For this the samples, immediately after they were taken, were diluted with minimum essential medium (MEM), with integral powers of ten being chosen as dilution factors. In each case 100 μl of a dilutes ample were transferred to 8 basins of a sterile polystyrene plate with a flat bottom. Then, in each case 100 μl of a freshly trypsinized suspension of KOP-R cells (cattle oesopharyngeal tissue, obtained from Dr R. Riebe, Bundesforschungsanstalt für Viruskrankheiten der Tiere auf der Insel Riems, Catalogue No. RIE 244) were added. This suspension was adjusted so that in each basin there were approximately $10–15 \times 10^3$ cells. Thereafter the samples were incubated at 37° C. in a CO_2 incubation cabinet (5% by volume CO_2). After 5 to 7 days, the infectious dose (ID_{50}/ml) was determined by the method of Spearmann-Kärber.

[0087] The virucidal activity was determined by calculating the decrease in titre compared with the respective control

titrations carried out in parallel (after the longest exposure time). The difference was reported as $\Delta\log_{10} ID_{50}$.

[0088] To determine the cytotoxicity of the disinfectant, 2 parts by volume of PBS (phosphate buffered saline) were mixed with 8 parts by volume of the disinfectant dilution (1.25-fold concentration), diluted correspondingly and applied to the cell cultures. The cytotoxic dose was reported as $\log_{10} CD_{50}$/ml (by analogy with the ID_{50} value).

[0089] The results of the inactivation tests are summarized in Table 2 hereinafter, and those of the cytotoxicity determination in Table 3.

TABLE 2

Concentration	Virus content (control) ($\log_{10}ID_{50}$/ml)	Protein load	Decrease in infection titre ($\Delta\log_{10}ID_{50}$) after 15 min	30 min	60 min	120 min
1.0%	6.85	—	1.25	1.25	1.64	n.d.
1.0%	7.05	0.2% BSA	1.00	1.25	1.34	n.d.
1.0%	7.15	10.0% FCS	1.00	1.15	1.25	n.d.
3.0%	7.65	—	≧4.15	≧4.15	≧4.15	≧4.15
3.0%	7.75	0.2% BSA	3.45	≧4.25	≧4.25	≧4.25
3.00%	7.65	10.0% FCS	2.17	3.45	≧4.15	≧4.15
5.0%	7.65	—	≧4.15	≧4.15	≧4.15	≧4.15
5.0%	7.75	0.2% BSA	4.05	≧4.25	≧4.25	≧4.25
5.0%	7.65	10.0% FCS	3.15	≧4.15	≧4.15	≧4.15

n.d. = not determined

[0090]

TABLE 3

Concentration	Dilution step 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
1.0%	+	-	-	-	-
3.0%	+	+	-	-	-
5.0%	+	+	-	-	-

[0091] The results show that the test composition (concentrate), at a usage concentration of 3.0%, after an exposure time of 60 min and at 5.0% after 30min has the effectiveness defined in the guideline ($\Delta\log_{10}ID_{50}$≧4.0; equivalent to an inactivation of ≧99.99%) towards ECBO viruses.

EXAMPLE 23

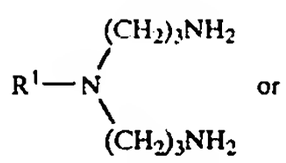
[0092] Effectiveness Against Canine Parvovirus:

[0093] Dilutions of the concentrate described in Example 20 were tested for their effectiveness against canine parvovirus type 2 (obtained from Dr Parrish, Cornell University) in NLFK cells (Norden Lab Feline Kidney) at 22° C. and an exposure time of 10 min.

[0094] Dilutions in a ratio of 1:35 in demineralized or hard (400 ppm AOAC hard water) water containing 5% organic load (foetal calf serum) showed adequate virucidal effectiveness.

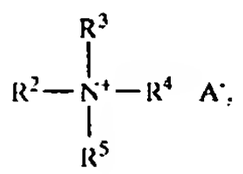
1. Use of a disinfectant composition comprising

a) an amine and/or quaternary ammonium salt of the general formula:



(Ia)

or



(Ib)

where R^1 is C_{6-18}-alkyl,

R^2 is benzyl or C_{6-18}-alkyl,

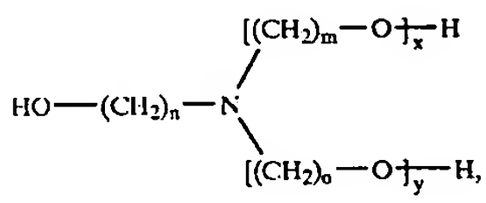
R^3 is C_{1-18}-alkyl or $-[(CH_2)_2-O]_nR^6$ where n=1-20,

R^4 and R^5 independently of one another are C_{1-4}-alkyl,

R^6 is hydrogen or unsubstituted or substituted phenyl,

and A^- is a monovalent anion or one equivalent of a polyvalent anion of an inorganic or organic acid; and

b) at least one alkanolamine of the general formula



(II)

where n and, if present, m and o independently of one another have the value 2 or 3,

and x and y independently of one another have the value 0 or 1, or a corresponding salt;

in the mass ratio I:II of 20:1 to 1:20 as virucidal agent.

2. Use according to claim 1, characterized in that the amine or quaternary ammonium salt is selected from the group consisting of N,N-bis(3-aminopropyl)dodecylamine, N,N-bis(3-aminopropyl)octylamine, didecyldimethylammonium salts, dioctyldimethylammonium salts, octyldecyldimethylammonium salts, cocoalkyldimethylbenzylammonium salts and benzyldimethyloxoethylammonium salts and mixtures of these compounds.

3. Use according to claim 1, characterized in that the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.

4. Use according to claim 1, characterized in that the mass ratio I:II is between 1:5 and 5:1.

5. Use according to claim 1, characterized in that the disinfectant composition comprises water as solvent.

6. Use according to claim 1, characterized in that the disinfectant composition additionally comprises one or more aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

7. Use according to claim 1, in surface disinfection and instrument disinfection.

8. Use according to claim 1, in laundry disinfection.

9. Use according to claim 1, in hand disinfection.

10. Use according to claim 1 in chemical toilets.

11. Use according to claim 1 against parvoviruses or picornaviruses, in particular against polioviruses.

12. Use according to claim 2, characterized in that the alkanolamine (II) is selected from the group consisting of monoethanolamine, diethanolamine, triethanolamine and 3-amino-1-propanol.

13. Use according to claim 2, characterized in that the mass ratio I:II is between 1:5 and 5:1.

14. Use according to claim 3, characterized in that the mass ratio I:II is between 1:5 and 5:1.

15. Use according to claim 12, characterized in that the mass ratio I:II is between 1:5 and 5:1.

16. Use according to claim 2, characterized in that the disinfectant composition comprises water as solvent.

17. Use according to claim 12, characterized in that the disinfectant composition comprises water as solvent.

18. Use according to claim 15, characterized in that the disinfectant composition comprises water as solvent.

19. Use according to claim 2, characterized in that the disinfectant composition additionally comprises one or more aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

20. Use according to claim 18, characterized in that the disinfectant composition additionally comprises one or more aids selected from the group consisting of organic solvents, surfactants, complexing agents, fragrances and colorants.

21. Use according to claim 2 in surface disinfection and instrument disinfection.

22. Use according to claim 20 in surface disinfection and instrument disinfection.

23. Use according to claim 2 in laundry disinfection.

24. Use according to claim 20 in laundry disinfection.

25. Use according to claim 2 in hand disinfection.

26. Use according to claim 20 in hand disinfection.

27. Use according to claim 2 in chemical toilets.

28. Use according to claim 20 in chemical toilets.

29. Use according to claim 2 against parvoviruses or picornaviruses, in particular against polioviruses.

30. Use according to claim 22 against parvoviruses or picornaviruses, in particular against polioviruses.

31. Use according to claim 24 against parvoviruses or picornaviruses, in particular against polioviruses.

32. Use according to claim 26 against parvoviruses or picornaviruses, in particular against polioviruses.

33. Use according to claim 28 against parvoviruses or picornaviruses, in particular against polioviruses.

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(10) RELATED PROCEEDING APPENDIX

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